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CONTENTS

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ENTOMON

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Dedicated to

Professor M.R.G.K. Nair

M.R.G.K. Nair
1922–1999

OBITUARY

Association for Advancement of Entomology (AAE) is sad to announce the death of Prof. M.R.G.K. Nair on 12th May 1999 at the age of 77. He has been the first President of AAE and held this position with distinction for over 24 years since the inception of the Association. In the December 1998 issue of ENTOMON, we reported on his retirement as President of AAE.

In the death of Prof. M.R.G.K. Nair, India has lost a leading Agricultural Entomologist. Learned, humble and unassuming, he held several positions with dignity and distinction. Born on 21st February 1922 at Muttambalam in Kottayam district of Kerala, he took his Masters degree in Zoology from the University of Lucknow and Associateship and Doctoral degree from the Indian Agricultural Research Institute, New Delhi. He began his professional career in the Agricultural Research Station at Pallom in Kerala in 1945. In 1946 he joined as Research Officer in Entomology in the University of Travancore. In 1955 he shifted to the newly established College of Agriculture at Vellayani in Kerala, where he became Professor of Entomology in 1963. He built up a full-fledged Department of Entomology in the College of Agriculture under the Kerala Agricultural University (KAU), with excellent facilities for teaching and research in biology, ecology, toxicology, insect pathology, biological control, storage entomology, nematology, etc. From 1972 to 1975, he served the Kerala Agricultural University as Director of Research, before returning to his parent Department as Professor and Head. After retirement in 1977, he served the University for another two years as an ICAR Emeritus Scientist.

Prof. M.R.G.K. Nair published over 90 scientific papers and guided 36 students for their M.Sc. degree and 8 students for Ph.D. degree. His books 'Crop Pests of Kerala and their Control' published by the Kerala Agricultural University and 'Insects and Mites of Crops of India', published by ICAR (revised in 1991) are authoritative accounts of the biology, ecology and control of agricultural pests and have won wide acclaim. He also published a book in Malayalam entitled "Karshikakeetavignanam" for which he won an All India Award from the University Grants Commission. He was a member of several distinguished Committees and Panels – ICAR Scientific panel, Executive Committee of KAU, Agricultural Expert in the Board of Directors of the State Bank of Travancore, etc. At the invitation of FAO and the World Bank he had organised training workshops on IPM for the benefit of Agricultural Officers of Sri Lanka.

Apart from academic pursuits, he was also active in social life, and gave philosophical discourses at various centres of the Sri Ramakrishna Mission, after retirement.

As the first President of AAE, he guided the fledgling Association and ably compensated for the untimely death of Prof. K.K. Nayar of the Department of Zoology of the University of Kerala at whose initiative the Association was established. After over 24 years of service to the Association, AAE in its annual General Body meeting

on 24th October 1998, accepted his request to relieve him as President of the Association. When I stepped into his shoes as President of AAE, I did not believe that his claim of failing health was real, and had hoped that his guidance will be available to us for many more years. But the inevitable happened rather unexpectedly- death overtook him in a matter of 6 months. He is survived by his daughter.

AAE places on record our deep indebtedness to him for the able guidance he provided us for almost a quarter of a century which has placed the Association on a sound footing. The example set by M.R.G.K, as we fondly called him, will continue to guide and inspire the AAE as well as scores of colleagues and students who had the opportunity to know and interact with him.

Dr. K.S.S. Nair
President, AAE

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Isolation and characterization of a hypoglycemic peptide from the brain of house fly *Musca domestica*

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ABSTRACT: Using a two step purification procedure involving gel filtration and reverse phase - high performance liquid chromatography (RP-HPLC) a hypoglycemic factor is isolated from the heads pooled from 9×10^4 house flies. Heterologous bioassays using cockroaches and frogs as test organisms revealed that the factor elicted a significant hypoglycemic effect. Dose - response studies showed that 6.25 head equivalents (Heq) of the extract purified by gel filtration decreased the hemolymph sugar levels of cockroaches by about 36% and 25 Heq by about 56%. The hypoglycemic effect became apparent 30 minutes after administration of the extract to the test organism and the effect lasted for about 90 minutes. The factor was stable at −20°C for several hours, at 37°C for a few hours. The water and methanol soluble factor lost its potency on treatment with trypsin and on oxidation with hydrogen peroxide. When injected into cockroaches the factor caused an increase in the protein content of the fat body and hemolymph but had no effect on the glycogen and lipid content of fat body and hemolymph as well as the protein content of the hemolymph. The amino acid composition of the hypoglycemic factor showed that the hydrophilic and hydrophobic amino acids constituted 66.27% and 33.73% respectively. The molecular weight of the isolated material as determined by gel permeation chromatography is approximately 2300.

KEYWORDS: *Musca domestica*, hypoglycemic factor, gel filtration, RP-HPLC, bioassay.

INTRODUCTION

The neuropeptides of insects that have been identified, isolated and their physiological role studied belong to four or five categories. They include peptides responsible for hyperglycemia or hypertrehalosemia, peptides which produce antidiuretic effect and peptides which promote myotropic activity (For review, see Holman *et al.*, 1988). Insect peptides appear to have extensive structural similarity among them but diverse physiological functions (O'shea *et al.*, 1984; Nagasawa *et al.*, 1986; Gade, 1989).

*Corresponding author

There are only a few reports of occurrence of hypoglycemic peptides in insects. Factors displaying immunological and biological activity similar to that of insulin have been reported in *Calliphora vomitoria* (Duve *et al.*, 1979). Hypotrehalosemic substances have been reported to occur in *Calliphora erythrocephala*, *Phormia regina* (Chen and Friedman, 1977) and *Manduca sexta* (Kramer *et al.*, 1980). The low molecular weight prothoracicotropic hormone of *Bombyx mori* known as 4 KD-PTTH II or *Bombyxin* has a striking 40% sequence homology with vertebrate insulin (Nagasawa *et al.*, 1984, 1986), although the hormone would not bind antibodies to insulin nor it would bind to insulin receptor. Recently Hetru *et al.* (1991) have isolated and elucidated the complete structure of an insulin like molecule from the corpora cardiaca of *Locusta migratoria*. In the present work a hypoglycemic peptide from the brain of *M. domestica* has been isolated, characterised and its biological activity tested in heterologous bioassays.

MATERIALS AND METHODS

Experimental organisms

House flies were cultured in the laboratory according to the procedure of Grady (1928) at 25 ± 2°C and 80 ± 5% rh. Adult cockroaches were maintained in the laboratory at 25 ± 2°C on a diet of soaked dog biscuits and water *ad lib*. Adult frogs (Rana hexadactyla) weighing 35 ± 2 g were maintained in the lab for a period of 7 to 10 days on a diet of pond snails.

Preparation of head extract of house flies

Heads severed from 9×10^4 house flies over a period of 3 months were stored at −20°C in an extraction solvent mixture of methanol, acetic acid and water (90 : 9 : 1 v/v). About 80 g of heads thus obtained were homogenised in a blender in excess quantities of extraction solvent mixture and small quantities of glass powder. The slurry was centrifuged at 10,000 g for 30 min. at 4°C to collect the supernatant. The residue was re-suspended in fresh extraction solvent, stirred well and centrifuged again. The supernatant was pooled with the earlier collection of supernatant in a siliconised glass container and evaporated at 30°C under vacuum to remove all methanol. After delipidation the extract was freeze dried.

Chromatography

Gel filtration of the lyophilized extract dissolved in glass distilled water (GDW) was carried out with Sephadex G 25 (fine) in a siliconised column (2.5 × 95 cm) using 1 M acetic acid as the eluent. 10 ml fractions were collected and absorbance read at 280 nm in a UV-vis spectrophotometer. The fractions were freeze dried, re-dissolved in GDW and were assayed for biological activity.

RP-HPLC of crude methanolic extract as well as the biologically active fractions obtained from gel filtration was carried out in Spherisorb (−18 (ISCO, USA) Column. The elutents were 0.11% aqueous trifluoroacetic acid (Solvent A, HPLC grade) and

60% acetonitrile (Sigma) in 1.0% trifluroacetic acid (solvent B). The column was eluted with a linear gradient of solvent A and B and the gradient range from 47 to 53%. The flow rate was 1 ml/min. One millilitre fractions were collected, freeze dried individually, re-dissolved in GDW and used for bioassays, amino acid analysis and molecular weight determination.

Bioassay procedures

Bioassay was performed to assess the hypoglycemic activity of the factor at each step of purification procedure. The dose is designated in terms of head equivalents (Heq). All control organisms received an equal amount of GDW. The sugar level of the hemolymph was determined after injection of the factor.

Characterization procedure

1. **Dose-response studies**
   From a concentrated aqueous extract containing 3000 Heq of the lyophilised fraction obtained by gel filtration, a series of dilutions ranging from 100 to 6.25 Heq were prepared. Batches of minimum 8 cockroaches were injected with 10 μl of a specific dilution. Sugar content of the hemolymph was estimated after 30 min. according to the modified anthrone method of Spik and Montreuil (1964) using glucose as standard.
2. **Time course studies**
   10 μl of head extract containing 25 Heq were injected into each of the 8 cockroaches. Prior to injections 5 μl of hemolymph was withdrawn from the cockroach, mixed with 0.75 ml concentrated sulphuric acid and stored at 4°C for determinations of sugar.
3. **Stability and solubility**
   Thermal stability of hypoglcemic factor was determined according to the procedure of Dores and Herman (1981). Four aliquots each containing 200 Heq of the hypoglycemic factor were left at −20°C for 12 h in a BOD incubator, at 37°C in an incubator for 6 h and in a boiling water for 5 min respectively. At the end of the incubation period the hypoglycemic activity was tested in cockroaches.
   The hypoglycemic factor was (i) incubated with trypsin (2 mg/ml) at 37°C for 1 h and (ii) oxidised in 0.1% H_2O_2 for 12 h at 20°C. At the end of the specified time the extracts were assayed for bioactivity in cockroaches.
   Solubility of the hypoglycemic factor was tested by preparing an extract of 2000 house fly heads separately in GDW, 100% methanol and chloroform. The heads were homogenized with an excess solvent which was subsequently evaporated *in vacuo* and gel filtered. The fractions are lyophilised, redissoved in GDW and assayed for biological activity in cockroaches.

Biochemical procedures

Analyses were carried out to test the effects of hypoglycemic factor in the metabolism of lipid, protein and glycogen. For such analyses haemolymph and fat body from a

TABLE 1. Sugar content (mg/ml) in hemolymph of control and hypoglycemic factor administered *P. americana*. The hypoglycemic factor was administered at different stages of purification

	Source	Concentration (Heq/10 μl)	Control	Experimental	%decrease
1.	Crude acidified methanolic extract	40	11.546 ± 0.363	6.625 ± 0.296	42.6*
2.	Gel filtered extract	25	12.135 ± 0.369	5.675 ± 0.302	53.2*
3.	Crude extract purified by RP-HPLC	15	11.685 ± 0.395	5.791 ± 0.360	50.4*
4.	Gel filtered extract purified by RP-HPLC	10	11.685 ± 0.395	5.784 ± 0.350	50.5*

Control insects received 10 μl of GDW. Sample size 8 for (1) and (2) and 6 for (3) and (4). For (3) and (4) control insects were common.

batch of 8 cockroaches each receiving 25 Heq of head extract were utilised. Protein was estimated according to the procedure of Lowry *et al.* (1951). Lipid was estimated following the method of Zollner and Kirsch (1962) and Folch *et al.* (1957). Glycogen was estimated according to the method of Carrol *et al.* (1956).

Molecular weight determination

Molecular weight of the hypoglycemic factor was determined by gel permeation chromatography following the method of Andrews (1965). Insulin, glucagon, cyanocobalamin, oxytocin and vasopressin (Sigma) were the standards used.

Amino acid composition

Amino acid composition of the hypoglycemic factor was quantitatively determined by HPLC according to the procedure of Rajendra (1987). The column derivatisation was done in O-phthalaldehyde (OPA) reagent. 20 μl of the sample was injected into the column. A standard amino acid mixture containing 21 amino acids was run in a similar way.

RESULTS

Chromatography

Figure 1 shows the elution profile of the acidified alcoholic extract of head of *M. domestica* in Sephadex G 25 (fine) column. The biological activity relates to the hypoglycemic effect in hemolymph in cockroaches. The fractionation of head extract yielded a major peak between fraction numbers 25 and 35 producing significant hypoglycemic activity when tested in cockroaches (Table 1). The elution volume (Ve) of the major peak constituted 62% of the total column volume (Vt).

Figure 2 shows RP-HPLC elution profile of crude acidified alcoholic extract of

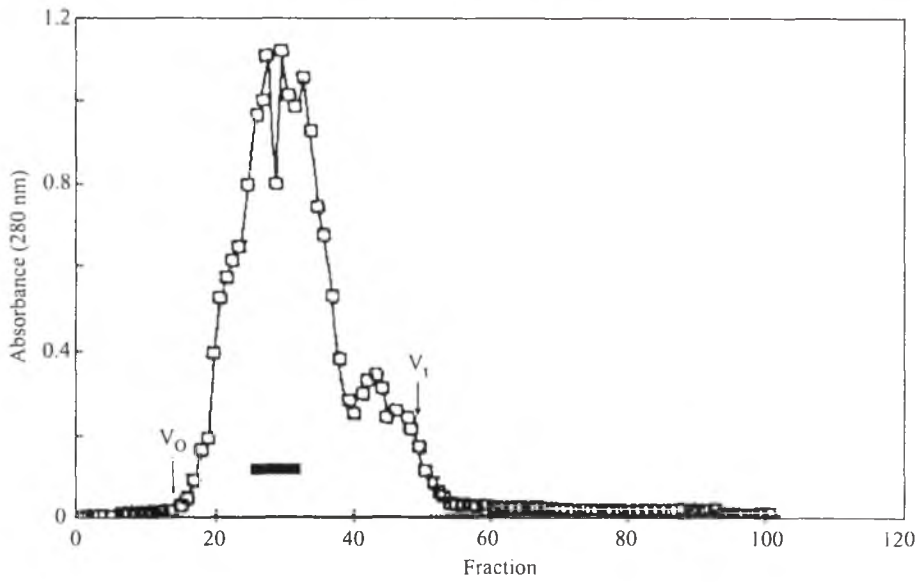

FIGURE 1. Elution profile of acidified methanolic extract of 9×10^4 heads of *M. domestica* subjected to gel filtration in a Sephadex G-25 (fine) column (2.5 × 95 cm). 1 M acetic acid pH 2.0 was the eluent used. Flow rate 40 ml/h. Fraction size 10 ml. V_o void volume: V_t total volume. Bar indicates fractions that showed bioactivity.

100 Heq. A large absorbance peak at the retention time of 3.29 min elicited a significant hypoglycemic response (Table 1).

Figure 3 shows RP-HPLC elution profile of head extract that was earlier passed through Sephadex G 25 column. A major peak with an area of 78% is the only fraction that elicited significant biological response in the test organism (Table 1). Table 1 also presents a comparison of hypoglycemic effect of the isolated factor at different levels of purification.

Characterization studies

1. **Dose-response curve**
   Figure 5 shows the relative potency of the hypoglycemic factor in cockroaches that received a dose of the factor ranging from 6.25 to 100 Heq. The dose-response curve shows that amount as low as 6.25 Heq produced a significant shift ($P < 0.01$) in hemolymph sugar levels. The maximum hypoglycemic activity was obtained at a dose of 25 Heq. Further increase in the amount of the isolated factor did not alter between the dosage injected and hemolymph sugar levels, the correlation coefficient being −0.965.
2. **Time course studies** The hypoglycemic activity was apparent 30 min after the injection of the head extract. The hemolymph sugar level dropped to 5.408 ±

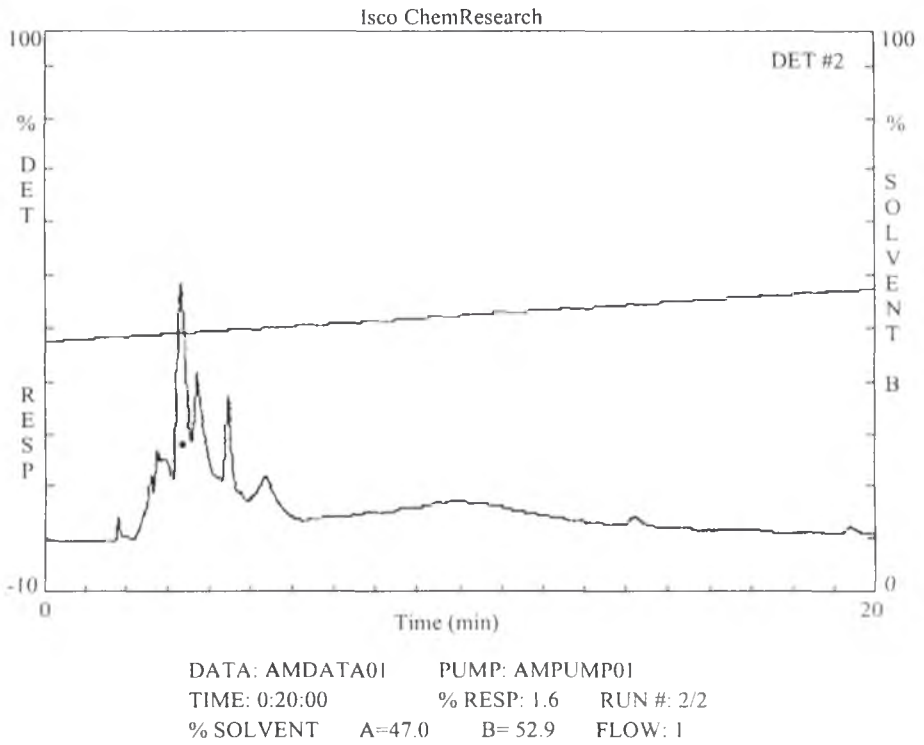

FIGURE 2. Separation of crude acidified methanolic extract from 100 heads of house flies using RP-HPLC on Spherisorb C-18 column. Absorbance at 280 nm; elution rate 1 ml/min; 20 min linear gradient from 47–53% acetonitrile in 0.1% TFA; •- Peak showing bioactivity.

0.55 mg/ml from the original value of 12.06 ± 0.469 mg/ml ($P < 0.01$). There was no further decrease in the sugar level, but the low levels were maintained for another 90 min before the normal sugar levels returned after about 120 min (Fig. 4).

3. **Stability and solubility studies** The hypoglycemic factor stored at −20°C for several weeks caused a 52% decrease in the sugar level of the hemolymph of the test organism ($P < 0.01$). More or less similar results were obtained in the test insects that were injected with brain factor stored at 20°C for 12 h or incubated at 37°C for 6 h. The potency was lost when the factor was kept in a boiling water bath for 5 min. The hypoglycemic factor was not functionally stable on hydrolysis by trypsin or on oxidation with hydrogen peroxide (Table 2). The factor is both water and methanol soluble but insoluble in chloroform. Injection of the factor prepared either in GDW or methanol into the cockroaches reduced the sugar levels of hemolymph by 52% ($P < 0.01$). But with chloroform as a solvent, sugar levels of hemolymph did not register any significant change (Table 2).

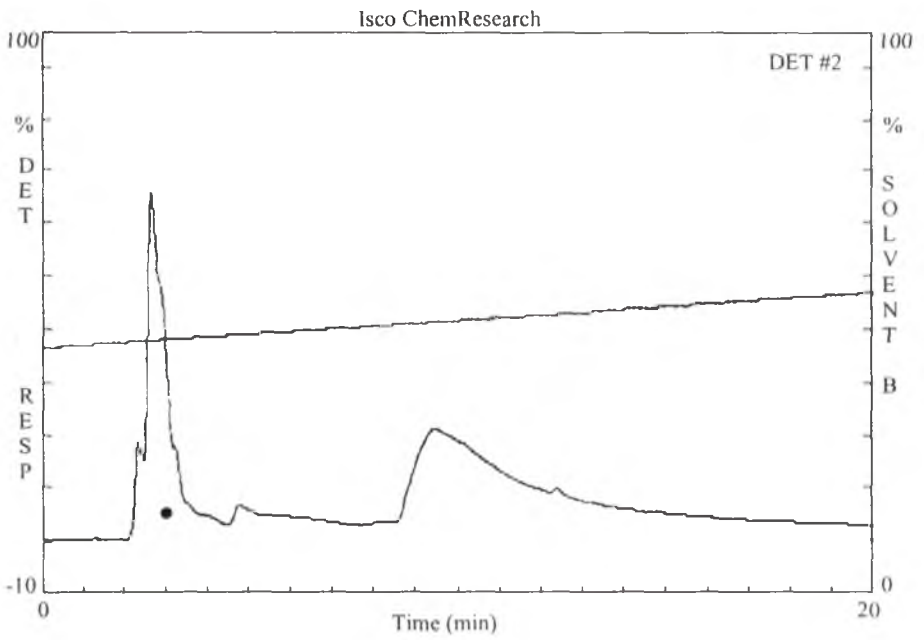

FIGURE 3. Separation of Sephadex G-25 bioactive fractions from 100 heads of house flies using RP-HPLC on Spherisorb C-18 column. Absorbance at 280 nm; elution rate 1 ml/min; 20 min linear gradient from 47–53% acetonitrile in 0.1% TFA; •- Peak showing bioactivity in *P. americana*.

TABLE 2. Solubility and stability properties of hypoglycemic factor of housefly. Sugar content of hemolymph expressed as mean ± SEM of 8 insects (For details refer to text).

1.	Solubility		
	Solvent	Sugar content (mg/ml)	% decrease
(a)	Control	12.163 ± 0.043	–
(b)	GDW	5.648 ± 0.388	53.6*
(c)	Methanol	5.510 ± 0.353	54.7*
(d)	Chloroform	12.034 ± 0.412	1.1
2.	Stability		
	Treatment	Sugar content(mg/ml)	% decrease
(a)	Control	12.163 ± 0.043	–
(b)	0.1%H_2O_2	12.447 ± .455	2.3
(c)	Trypsin	12.367 ± 0.479	1.7

Control insects received GDW * $P < 0.01$

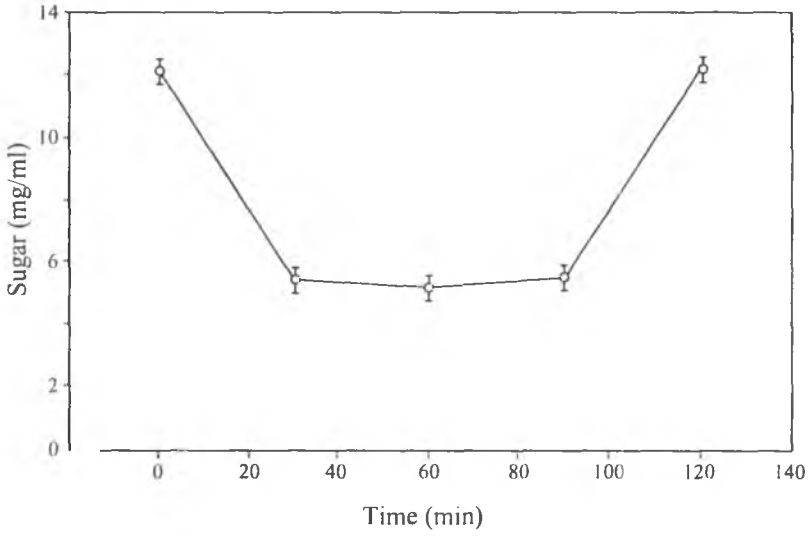

FIGURE 4. The time-course of hypoglycemic effect of the brain factor on hemolymph sugar concentration in *P. americana*. Each value is a mean ± SEM of 8 insects.

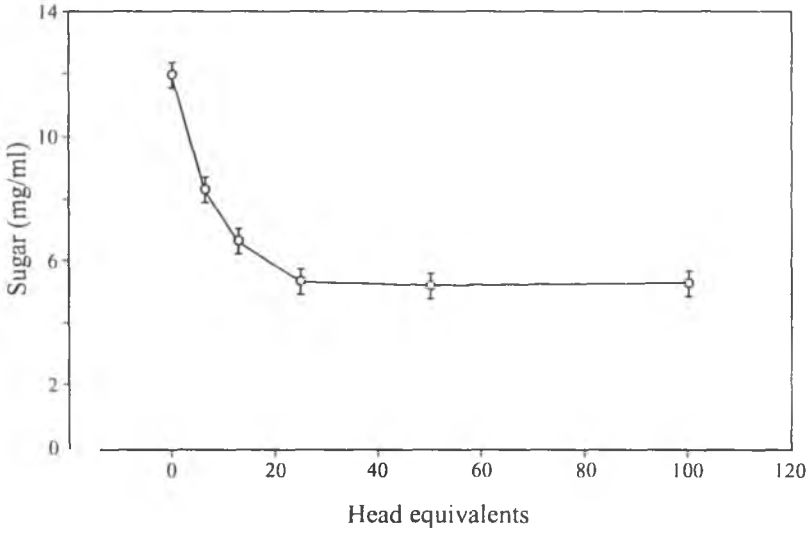

FIGURE 5. Dose-response curve of the hypoglycemic factor on hemolymph sugar concentration (mg/ml) in *P. americana*. Each value represents the mean ± SEM of 8 insects.

TABLE 3. Effect of administration of hypoglycemic factor on lipid, glycogen and protein content of the tissues of *P. americana*. Each value is a mean ± SEM of 8 determinations.

		Control	Experimental	% difference
(a)	Lipid			
	Hemolymph (mg/ml)	8.5 ± 0.112	8.202 ± 0.85	−3.4
	Fatbody (μg/mg)	39.7 ± 2.3	41.9 ± 2.1	+5.6
(b)	Glycogen			
	Whole animal (mg/g)	2.003 ± 0.07	2.2 ± 0.06	+8.9
	Fatbody (μg/mg)	21.48 ± 2.0	17.9 ± 0.26	−16.6
(c)	Protein			
	Hemolymph (mg%)	50.67	49.33	−2.6
	Fatbody (μg/mg)	3.601	11.27	213*
	Midgut (μg/mg)	1.807	4.235	134*

Control insects received GDW * $P < 0.01$

Metabolic effects

The hypoglycemic factor neither altered the lipid content of hemolymph and fat body nor the total glycogen of the body changed (Table 3). The protein content of hemolymph was not altered by the isolated factor whereas the midgut and fat body protein concentrations did register a significant increase ($P < 0.01$) (Table 3).

There was a pronounced hypoglycemic effect when the frogs received 50 Heq of the house fly brain factor intraperitoneally. The blood sugar level was brought down by 50% as compared to 35.7% reduction in blood sugar level caused by the administration of 2 units of insulin. Further, insulin injected frogs recorded a 48% increase in blood protein levels as compared to control animals and in frogs that received the house fly brain factor there was a 112% increase in blood protein concentration.

Amino acid composition and molecular weight

The RP-HPLC amino acid profile of the hypoglycemic factor is presented in Table 4. Thirteen amino acids have been identified and quantified. Tyrosine is the major component followed by leucine and glutamate. Hydrophilic amino acids gly, gln, tyr, asp, glu, arg and lys constituted 66.27% of the hypoglycemic factor and hydrophobic amino acids ala, ile, leu, met, phe and val constituted 33.73%.

TABLE 4. RP-HPLC analysis of amino acid composition and quantification (%) of the hydrolysate of HPLC purified hypoglycemic factor.

Amino acid	%
Aspartate	1.64
Glutamate	17.46
Glutamine	3.93
Glycine	4.79
Arginine	9.51
Alanine	13.22
Tyrosine	21.04
Methionine	Not quantified
Valine	3.76
Phenylalanine	3.86
Isoleucine	0.40
Leucine	12.49
Lysine	7.90

DISCUSSION

Although a number of substances that have hypoglycemic effect have been identified in insects, their physiological role appears to be different in different insects (Tager *et al.*, 1976; Chen and Friedman, 1977; Duve *et al.*, 1979; Loughton, 1987). There are only a few reports of insect hormones producing a hypoglycemic effect despite the fact that a large number of insulin like peptides have been identified. In *L. migratoria* an insulin like molecule from the midgut functions as a hypoglycemic factor (Moreau *et al.*, 1982). Glucagon and insulin like peptides with designated biological activity are reported to be present in *Manduca sexta* (Tager *et al.*, 1976). In *C. vomitoria* the occurrence of an insulin like molecule that displaced insulin from rat liver cell plasma membrane insulin receptors and exhibited insulin like bioactivity in the isolated fact cells of rat is reported (Duve *et al.*, 1979). In addition insulin like material is reported to occur in the larval food of honey bee (Dixit and Patel, 1964) and in various tissues and body fluids in hymenoptera (Ishay *et al.*, 1976).

The hypoglycemic factor isolated from the brain of house flies by a two step purification procedure caused a significant decrease of the sugar concentration of the hemolymph of cockroaches and enhanced the protein levels, the two physiological roles attributed to insulin. Considering the size of the head, more specifically that of the brain, as well as the fact that the material is synthesized and stored transiently in the cells, the factor was obtained from as large number of insects as possible. The ring gland, a storage organ for other peptides as well, was carefully excluded while severing the heads to avoid the possible mixing with other secretions. And based on the

histological evidence that the stainable material is present in appreciable quantities in 3 to 5 day old adults, (Marcus, 1994) flies of corresponding age were used in extraction.

Gel permeation chromatography was used to exclude all components whose molecular weight exceeded 5000 daltons. Nevertheless such fractions were also tested for bioactivity, but only negative results were obtained. The biological activity was confined to fractions 25 to 35, each of 10 ml. RP-HPLC which makes use of hydrophobic interactions (Gade, 1988) was adopted for the purification of the peptide. Fractions collected at the retention time of 2.29 min exhibited biological activity. The brain factor of house fly is essentially a highly polar and a low molecular weight peptide as is evidenced by the early elution of the factor in RP-HPLC. The biological activity is expressed in terms of head equivalents consistently. The threshold concentration required of crude acidified methanolic extract to biological response was 40 Heq. On further purification, relatively less and less concentrations were required to produce a more or less similar hypoglycemic effect. Thus 25 Heq were sufficient after gel filtration, 15 Heq of HPLC purified crude extract and 10 Heq of gel filtered and HPLC purified extract.

The dose-response curve was an inverse hyperbola. The maximum hypoglycemic activity was obtained at a concentration of 25 Heq, although the lower concentrations of 12.5 Heq and 6.25 Heq did elicit a significant ($P < 0.01$) hypoglycemic response. Further increase in the concentration of hypoglycemic factor did not significantly alter the sugar concentration of hemolymph. Essentially a dose of 25 Heq effects a stabilisation of sugar levels. The efficiency of the hypoglycemic factor at low concentrations suggests a physiological role for the peptide. Over a concentration range of 6.25 Heq and 25 Heq a high negative correlation (-0.965) existed between the concentration injected and the hypoglycemic effect produced. The maximum effect was apparent 30 min after the injection of the extract and the effect lasted another 60 min. Normal sugar levels were restored in hemolymph nearly two hours after the administration of the extract. In *C. vomitoria* the hypotrehalosemia produced by the insulin like material persisted for about 2 h (Duve *et al.*, 1979). In *L. migratoria* the insulin like molecules from the midgut did induce hypotrehalosemia, but the effect was short lived for about 15 to 30 min (Moreau *et al.*, 1982). The hypoglycemic factor is both water and methanol soluble and insoluble in chloroform.

The biological activity of the hypoglycemic factor was fully conserved at various temperatures at which it was stored except at very high temperatures. The material stored at different temperatures did not exhibit any loss of activity which meant that the injection of a concentration of 25 Heq in the test organism resulted in the expected hypoglycemic effect. The locust antidiuretic hormone showed a striking stability even after heated to 100°C (Fournier *et al.*, 1987). Contrarily the diuretic factor from the same insect showed poor stability at 25°C. There was no loss of biological activity when the hormone was heated to 70°C and stored at 23°C for 24 h suggesting that at 25°C the hormone was degraded by enzymes released during the sonication procedure (Morgan and Mordue, 1983). The hypoglycemic factor from house flies is degradable by trypsin or on oxidation with H_2O_2.

The metabolic effects of the injected hypoglycemic factor in the test organism relate to significant reduction in the sugar levels of hemolymph and enhanced protein levels of midgut and fat body. There was no change either in the lipid content of the hemolymph and fat body or in the glycogen levels of fat body and the whole insect. More interestingly the hypoglycemic factor of the house flies caused a significant reduction in the blood sugar levels of frogs. In producing these effects the factor mimics the action of insulin. The decrease in sugar levels did not cause a concomitant increase in the glycogen levels of cockroach. In *Phormia regina*, the disappearance of the trehalose from the hemolymph due to the administration of the hypotrehalosemic factor from brain is attributed to the increase in blood osmotic pressure through accelerated diuretic activity (Chen and Friedman, 1977). As far as the increased level of proteins in the midgut and fat body, it is known that in vertebrates insulin does promote the uptake of branched chain amino acids valine, leucine and isoleucine by muscles and favour building up of muscle proteins. In addition it inhibits intracellular digestion of proteins (Orchard and Loughton, 1980). In *M. sexta* an aqueous extract from CC-CA complex produced both glycogenolysis and hypoglycemia when injected into the larvae of same species suggesting the occurrence of insulin like glucagon like peptides, and the peptides find their way to the target tissues through hemolymph (Kramer *et al.*, 1980).

Thirteen amino acids were identified by RP-HPLC of the acid hydrolysate of the hypoglycemic factor. Tyrosine occurred in larger quantities followed by glutamic acid, alanine, leucine and arginine. The OPA method adopted for amino acid analysis is insensitive to amino acids proline and hydroxy proline although the TLC plates did reveal an yellow spot. The occurrence of tyrosine in large quantities accounts for strong absorbance at 280 nm. The molecular weight of the hypoglycemic factor as determined by gel permeation chromatography was in the range of 2200–2300 daltons. A positive immunoreactivity of the neurosecretory material was observed when the median neurosecretory cells of house fly were incubated with monoclonal anti-somatomedin C (insulin like growth factor-I) in sections and whole brain mounts (Marcus and Raghavan unpublished) Somatomedin C is single chain peptide with 70 amino acids and the sequence is similar to proinsulin.

In future more attention should be given to the primary sequencing of hypoglycemic factor which will throw light on the relatedness or otherwise of the resemblance to insulin. Studies in related insects would also reveal whether a family of hypoglycemic peptides exist.

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New Host Records and a New Species of Tenuipalpid Mites Infesting Deciduous Fruit Trees in Punjab, India

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ABSTRACT: Nine species of tenuipalpid mites viz. *Brevipalpus phoenicis* (Geijskes), *B. euphorbiae* Mohanasundaram, *Tenuipalpus kesari* Sadana, Gupta and Goyal, *T. micheli* Lawrence, *T persicae* Sadana, Chhabra & Gupta, *T. pyrusae* Maninder and Ghai, *T. acuminatae* Mohanasundaram, *Tenuipalpus* species and *Priscapalpus gurdaspurensis* sp. nov. inhabiting deciduous fruit trees are reported from the Punjab State. Out of these, four mite species are reported on new host plants i.e. *Brevipalpus phoenicis* (Geijskes) on phalsa, *B. euphorbiae* Mohanasundaram on pear and ber, *Tenuipalpus persicae* Sadana, Chhabra and Gupta on pear and *T. micheli* Lawrence on peach and pear. *Brevipalpus euphorbiae* Mohanasundaram and *Tenuipalpus micheli* Lawrence are reported for the first time on deciduous fruit trees. A new species of the genus *Priscapalpus* DeLeon is described and illustrated.

KEYWORDS: Tenuipalpid mites, deciduous fruit trees, Punjab, new species, new hosts).

INTRODUCTION

The tenuipalpid mites are of great economic importance as they infest almost all types of plants including fruit trees (Meyer, 1979; Ghai and Shenhmer, 1984; Sadana, 1985, 1997; Sadana and Chhabra, 1980) and bring about economic losses. Inspite of their great economic importance and common occurrence, very little efforts have been made to explore the mite fauna infesting deciduous fruit trees in the Punjab State. Whatever little we know about them is due to the work of Gupta *et al.* (1971); Sadana and Gupta (1982); Sadana and Sidhu (1990); Sadana and Kaur (1992). With this view in mind, we made extensive surveys of Punjab state to record tenuipalpid mite species infesting deciduous fruit trees. As a result a new tenuipalpid mite and many new host records were made which are reported here in.

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MATERIALS AND METHODS

A large number of mites were collected from deciduous fruit trees namely pear, *Pyrus communis* L.; peach, *Prunus persica* Stokes; plum, *Prunus salicina* L.; ber, *Zizyphus mauritiana* Lamk.; grapes, *Vitis vinifera* L.; guava, *Psidium guajava* L.; mulberry, *Morus alba* L. and phalsa, *Grewia asiatica* L. The infested leaves were brought to the laboratory in separate polythene bags. Each bag was tightly closed with a rubber band to prevent contamination. At the time of collection, a slip containing information about host, locality, date of collection, collector etc. was placed in each polythene bag. The mites were isolated from collected material with Berlese's method in case the infestation was heavy, otherwise these were picked up individually with the help of zero numbered camel's hair brush under stereoscopic binocular microscope. The mites thus isolated were cleared in lactophenol and then mounted on slides in Hoyer's medium. The slides prepared were dried in an oven maintained at 37°C and ringed with ordinary nail polish. The detailed study of mites was made under oil immersion of compound binocular microscope.

RESULTS AND DISCUSSION

In all, nine tenuipalpid mite species were encountered during the present investigations. Of these, two mite species i.e. *Tenuipalpus micheli* and *Brevipalpus euphorbiae* are reported for the first time on deciduous fruit trees. The following is the list of mites along with their hosts and distribution records. The new host records have been marked with asterisks (*) in the collection data. The new species recorded during the present work is described and illustrated. The measurements given are in microns.

1. *Brevipalpus phoenicis* (Geijskes)

Collection data: 4 females: India: Gurdaspur, ex. *Vitis vinifera*, 25.V.97, Coll. Mrs. Surinderjit & Ms. Manjit; 1 female, Ludhiana, Punjab Agricultural University, ex. *Grewia asiatica**, 15. IX.97, Coll. Mr. Rabinder; 1 female, Jallandhar, ex. *Pyrus communis*, 20.VI.97, Coll. Mr. Kulwant Singh.

2. *Brevipalpus euphorbiae* Mohanasundaram

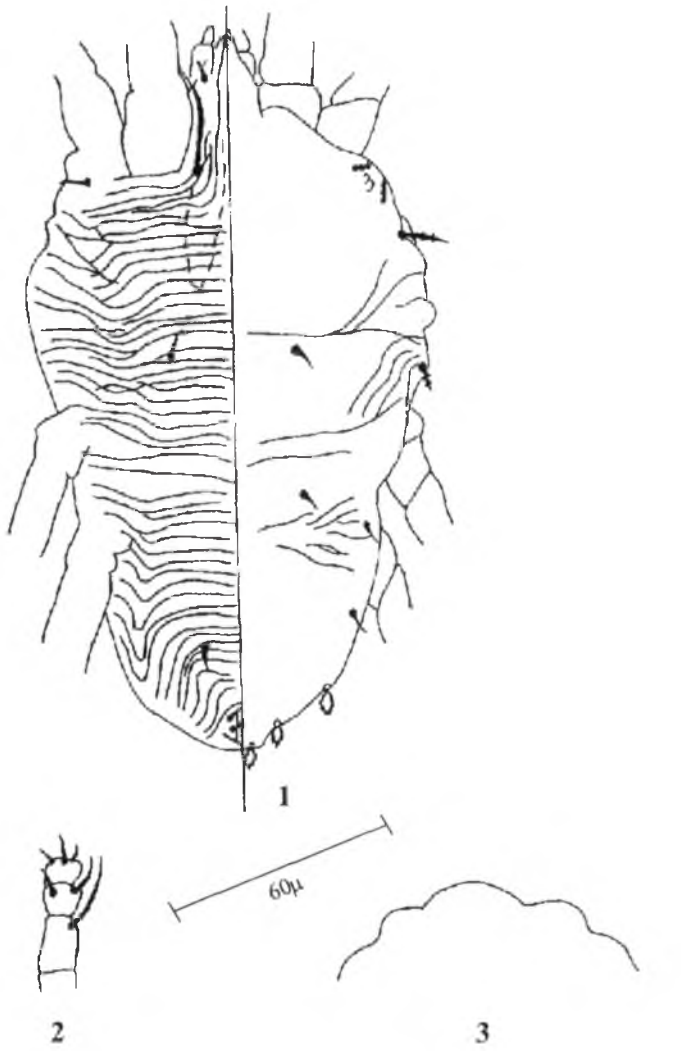
Collection data: 1 female: India: Hoshiarpur, ex. *Zizyphus mauritiana**, 15.VII.97, Coll. Mr. Kulwant Singh; 1 female, Patiala, ex. *Pyrus communis**, 20.VI.97, Coll. Mr. Rabinder.

3. *Tenuipalpus kesari* Sadana, Gupta & Goyal

Collection data: 3 females: India: Hoshiarpur, ex. *Pyrus communis*, 13.VII.97, Coll. Randeep.

4. *Tenuipalpus micheli* Lawrence

Collection data: 1 female: India: Ludhiana, ex. *Prunus persica**, 27.V.96, Coll. Randeep; 1 female, Jalandhar, ex. *Pyrus communis**, 5.V.97, Coll. Mrs. Surinderjit.



FIGURES 1–3. *Priscapalpus gurdaspurensis* sp. nov. 1: Dorsal view of female (right half) and Ventral view of female (left half) 2: Palpus 3: Rostral shield.

5. *Tenuipalpus persicae* Sadana, Chhabra & Gupta

Collection data: 1 female: India: Patiala, ex. *Pyrus communis**, 4.VI.97, Coll. Mr. Paramjit; 1 female, Bathinda, ex *Pyrus communis*, 20. VI. 97, Coll. Mr. Rabinder; 1 female, Gurdaspur, ex. *Pyrus communis*, 4.VII.97, Coll. Mrs. Surinderjit.

6. *Tenuipalpus pyrusae* Maninder & Ghai

Collection data: 1 female: India: Ludhiana, ex. *Pyrus communis*, 5.VI.97, Coll. Randeep.

7. *Tenuipalpus* sp.

Collection data: 1 female: India: Jalandhar, ex. *Pyrus communis*, 13.X.96, Coll. Mrs. Surinderjit.

8. *Tenuipalpus acuminatae* Mohanasundaram

Collection data: 1 nymph: India: Hambran (Ludhiana), ex. *Prunus persica*, 29.V.96, Coll. Randeep.

9. *Priscapalpus gurdaspurensis* sp. nov.

Female: Body 165 long including gnathosoma and 115 wide. Rostral shield with a median rounded lobe and a lateral lobe on each side. Palpus 4 segmented, second segment longest and provided with a seta, third segment with a pair of setae while terminal segment with a long sensory rod and a pair of setae. Propodosoma with a few oblique lines posterolaterally. Propodosomal setae 3 pairs, serrate measuring 7.2, 7.2 and 15.6 long, respectively. Humeral setae one pair, serrate measuring 9.6. Hysterosoma with a few transverse lines. Dorsocentral setae 2 pairs measuring 4.8 and 3.6 long, respectively. Dorsolateral setae 5 pairs measuring 6, 7.2, 12, 14.4 and 15.6 long, respectively. 3rd, 4th and 5th pairs spatulate and serrate while first two pair of dorsolateral setae small and simple. Gnathosoma with a pair of setae on venter. Medioventral propodosomal setae one pair extending upto middle of second palpal segment. Anterior medioventral metapodosomal setae one pair measuring 7.2. Genital setae one pair, anal setae 2 pairs. All setae on ventral side simple. Venter of propodosoma and hysterosoma with transverse striations. Legs 4 pairs.

Male: Not known

Holotype: 1 female (marked on slide No. G-3) India: Punjab, Gurdaspur, ex. *Vitis vinifera* L., 20.VI.97, Coll Mrs. Surinderjit.

Paratype: 1 female (slide No. P-13), Punjab, Patiala, ex. *Pyrus communis*, 15.VI.97, Coll. Randeep.

Repository: The holotype and paratype are housed in the Acarological collections of the Department of Zoology, Punjab Agricultural University, Ludhiana but will be deposited in the National Pusa Collection, I.A.R.I., Delhi in course of time.

Remarks: The present species shows slight resemblance with *Priscapalpus cherreti* DeLeon but it differs widely from it and all other species described under this genus in the following features. There are no blunt knobs anterior to coxa III and palpus is four segmented instead of one segment as in *P. cherreti*. It further differs from other species of genus *Priscapalpus* in having spatulate and serrate 3rd., 4th and 5th pairs of dorsolateral setae. While first two pair of dorsolateral setae are simple and small.

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Neurotoxicity of Synthetic Pyrethroids, Fenvalerate and Cypermethrin in the Cockroach, *Periplaneta americana*

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ABSTRACT: The spontaneous electrical activity of the abdominal nerve cord decreased remarkably in the cockroach, *periplaneta americana* following bath application of the synthetic pyrethroids, cypermethrin and fenvalerate. Sensory mediated giant - fibre responses in the nerve cord to cercal stimulation by air puffs almost disappeared in the cockroaches topically exposed to lethal doses of fenvalerate and cypermethin while the sublethal doses produced a marginal decrease in giant fibre responses. These changes were attributed to the possible damage to the cercal mechano sensory system due to the action of pyrethroids.

KEYWORDS: Cockroach, fenvalerate, cypermethrin, neurotoxicity

INTRODUCTION

Pyrethroids exert widespread, potent actions on both peripheral and central nervous elements in arthropods. Electrophysiological studies have revealed important features of action of pyrethroids at cellular and subcellular levels. (Burt and Goodchild, 1971; Leake, 1977; Narahashi and Lund, 1980).

The neurophysiological responses to pyrethroids vary according to the type of nerve element studied, the compound applied and the temperature. Motor terminals and sensory structures were found to be more sensitive to pyrethroids than unspecialised axonal membranes (Clements and May, 1977). Interactions of fenvalerate with nerve membrane sodium channels and block of conduction were reported by Holloway *et al.* (1989). Vijverberg and van den Bercken (1990) have proposed that the primary target sites of pyrethroids are the voltage dependent sodium channels. Present study was aimed at studying the action of two different pyrethroids, fenvalerate and cypermethrin on the spontaneous electrical activity and giant fibre responses to cercal stimulation in the ventral nerve cord of the cockroach, *Periplaneta americana* to understand the changes in sensory and interneuronal transmission.

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MATERIALS AND METHODS

Adult male cockroaches purchased from local animal supplier were acclimated to the laboratory conditions (25°C to 35°C) for one week in wire mesh cages before using them for experiments. They were fed *ad libitum* on bread and rice on a fixed feeding time schedule. Feeding was stopped one day before the commencement of the experiment to avoid any metabolic variations due to diet. Only adult male cockroaches of approximately same size were used for the experiments, since females were demonstrated to exhibit "erratic" rhythmicity (Roberts and De, 1960) and influenced by the state of reproductive cycle (Leuthold, 1966; Liption and Sutherland, 1970).

Commercial grade synthetic pyrethroids fenvalerate 20 EC (Rallies India Ltd., Bombay) and cypermethin 10 EC (SPIC Ltd., Madras) were used for both bath application of nerve cord and topical application to cockroaches. A 24 hour LD_{50} was determined using the "probit method" (Finney, 1964) and 1/3 of LD_{50} was considered as sublethal dose.

Electrophysiological studies

The animals were fixed to a wax filled dissection board with the dorsal side up. The dorsal abdominal body wall was removed to expose the ventral nerve cord (VNC). Care was taken to see that no gut contents spilled over the VNC. Electrophysiological recordings were made in air, at room temperature (28°–32°C).

Spontaneous electrical activity of control animals was recorded for 10 minutes to confirm whether there was sustained activity throughout the period. The portion of the connective under which the electrodes were placed was covered with a drop of liquid paraffin to avoid drying of the connectives.

Similar procedure was followed to test the effects of pyrethroids (fenvalerate and cypermethrin). Both lethal and sublethal concentrations of pyrethroids were tested for their effect on spontaneous activity as well as giant fibre responses to the cercal sensory stimulation with controlled air puffs. To examine the giant fibre responses, the cercal sensilla were stimulated with a mechanically generated controlled air puff.

The signals from VNC were amplified with P_5 Grass AC preamplifier and fed simultaneously to Systronics 5100, dual beam oscilloscope and a tape recorder. Grass C_4 Kymograph camera was used to record the signals from oscilloscope on the photographic film. The tape recorded information was fed to the 4-digit counter and oscilloscope to count the number of spikes per minute. The spike counts were also checked manually and presented as number of spikes/sec.

RESULTS AND DISCUSSION

The spontaneous electrical activity recorded at 08.00 pm. in control animals was 50 to 80 spikes/sec. Bath application of sublethal doses of fenvalerate reduced the activity within two minutes and the activity remained more or less same in the remaining eight minutes of recording time. The spontaneous activity (number as well as the amplitude of spikes) greately reduced when the sublethal dose of fenvalrate was replaced by

TABLE 1. Changes in spontaneous electrical activity of VNC of cockroach, *Periplaneta americana* following perfusion with cypermethrin and fenvalerate (activity is represented in spikes/sec).

Name of pyrethroid	Control	Sublethal dose	Lethal dose	Recovery
Fenvalerate	50	42	33	41
PDC	–	−16	−34	−18
Cypermethrin	80	60	49	66
PDC	–	−25	−38.5	−17.5

PDC = Percent Deviation over Control

lethal dose. The electrical activity however showed partial recovery from the effect of lethal dose when the VNC was subjected to ringer wash for 5 minutes. Sublethal and lethal doses of cypermethrin also produced similar decrease in VNC activity but the decrease in activity with both the concentrations was marginally higher than fenvalerate. (Table 1).

Mechanical stimulation of abdominal cercus with controlled air puffs produced burst of activity in the VNC of control animals (Fig. 1). Cockroaches exposed topically to fenvalerate or cypermethrin, however, failed to elicit evoked potentials as that of controls. Animals exposed to sublethal doses of fenvalerate and cypermethrin though showed responses to cercal stimulation, the number and amplitude of spikes decreased considerably indicating that some giant fibres have not responded. Lethal doses on the otherhand produced complete loss of activity (both spontaneous as well as sensory mediated giant fibre activity) (Fig. 1) excepting occasional firing of one or two giant axons. Between the two pyrethroids, cypermethrin seems to have damaged the cercal receptor system to a greater extent than fenvalerate as revealed from the number and the amplitude of spikes recorded from the VNC.

The recovery observed in the spontaneous activity of VNC with a ringer wash, suggests that the effect of pyrethroids on nerve action potentials is reversible even at higher concentrations provided the effect is countered shortly after application. The cercal mechano sensory system failed to evoke giant fibre (GI) responses in the VNC to the air puff stimuli in *P. americana* following topical application of lethal doses of cypermethrin or fenvalerate. Sublethal doses, on the otherhand were still effective, if not to the threshold level, in eliciting the giant fibre responses to cercal stimulation. These observations strongly suggest that the topical application of pyrethroids at high concentrations cause damage to the sensory structures and block the sensory mediated giant fibre responses in cockroach.

In addition to their well characterised effect on nerve axons, pyrethroids have been known to affect other neuronal elements, especially sensory structures. Gammon (1978) reported that allethrin-treated cockroaches developed bursts of afferent discharges in the cerci because the animals became restless. Locusts treated topically

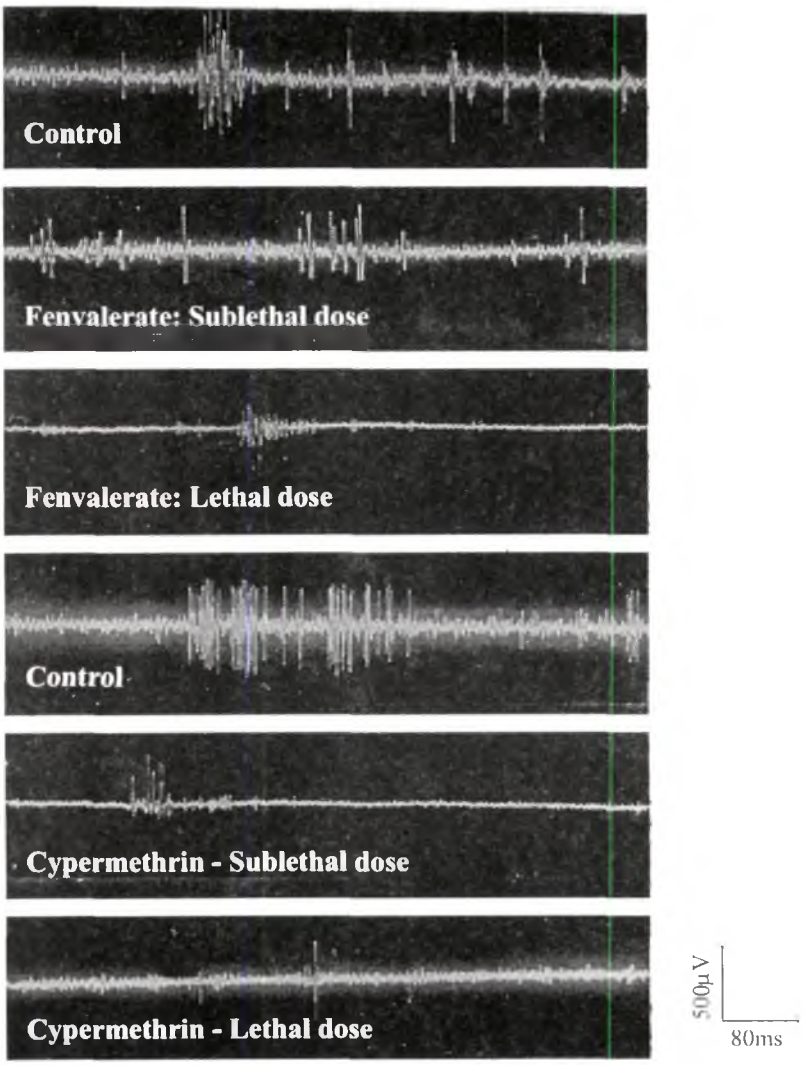

FIGURE 1. Effect of the topical application of the pyrethroids fenvalrate and cypermethrin on the cercal sensory responses in the ventral nerve cord of cockroach, *Periplaneta americana*.

with pyrethroids showed bursts of afferent activity coinciding with the knockdown stage (Clements and May, 1977). All these observations including the findings of the present study, strongly suggest that pyrethroids act directly on the sensory structures when applied topically, since the ablation of the sensilla removed the effect although the sensory axons were left intact. The disapperence of giant spikes (the response of GI's to cercal stimulation) in the cockroaches treated with lethal doses of pyrethroids

suggests that the cercal sensilla were damaged enough that stimulation could not elicit a response. The partial responses with decrease in the number and amplitude of spikes in the giant fibres of the VNC in the animals topically exposed to sub lethal doses, on the other hand, suggest that the lower concentrations may not cause much damage to the sensory system. Among the two pyrethroids, the cypermethrin produced more inhibition in both spontaneous activity as well as cercal sensory evoked GI responses in the VNC indicating its greater toxicity.

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Reporting of a New Species Under Genus *Creatonotos* Hübner (Arctiinae: Arctiidae: Lepidoptera) from India

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ABSTRACT: A new species *ananthakrishanani* under genus *Creatonotos* has been described and illustrated. This species is closely allied to *transiens* (Walker) as far as its wing maculation is concerned. The species also conform to the type species *interruptus* (Linnaeus) of the present genus. However, the new species can be easily distinguished on the basis of its distinct genitalic structures from the other two species. A key to the Indian species of the genus has also been erected.

KEYWORDS: *Creatonotos*, species and genitalia.

INTRODUCTION

While conducting surveys in North-West India for the collection of Arctiid moths, as many as thirteen adult representatives of a species referable to genus *Creatonotos* Hübner have been collected from different localities of Himachal Pradesh, Jammu and Kashmir and Uttar Pradesh. This species could not be identified from the relevant literature (Hampson, 1894; Arora and Choudhary, 1982; Holloway, 1988; Kishida *et al.*, 1992) and by comparison from three National Museums as well as from the Natural History Museum, London. The comparison was done with the help of David T. Goodger, an eminent Lepidopterist of Natural History Museum, London. The detailed morphological studies along with the examination of male and female genitalic structures revealed that though congeneric, this new species is distinct from all other known species of genus *Creatonotos* Hübner on the basis of its unique features. The male and female genitalia of the type species *interruptus* (Linnaeus) and *transiens* (Walker) has already been described by Kirti and Amritpal Singh (1994). Role of genitalia in family Arctiidae has been explained by Kirti and Amritpal Singh in 1996.

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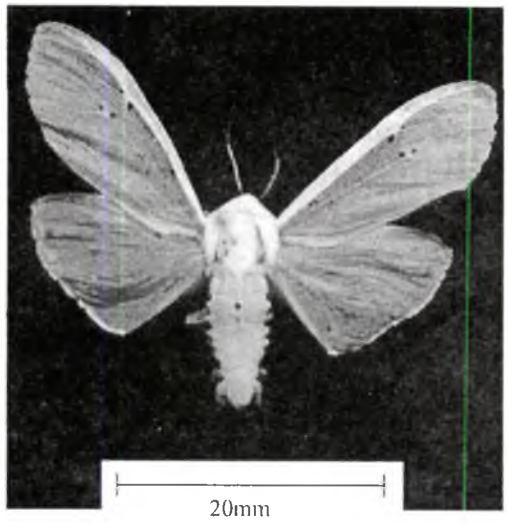


FIGURE 1. Adult of *Creatonotos ananthakrishanani* n. sp.

OBSERVATIONS

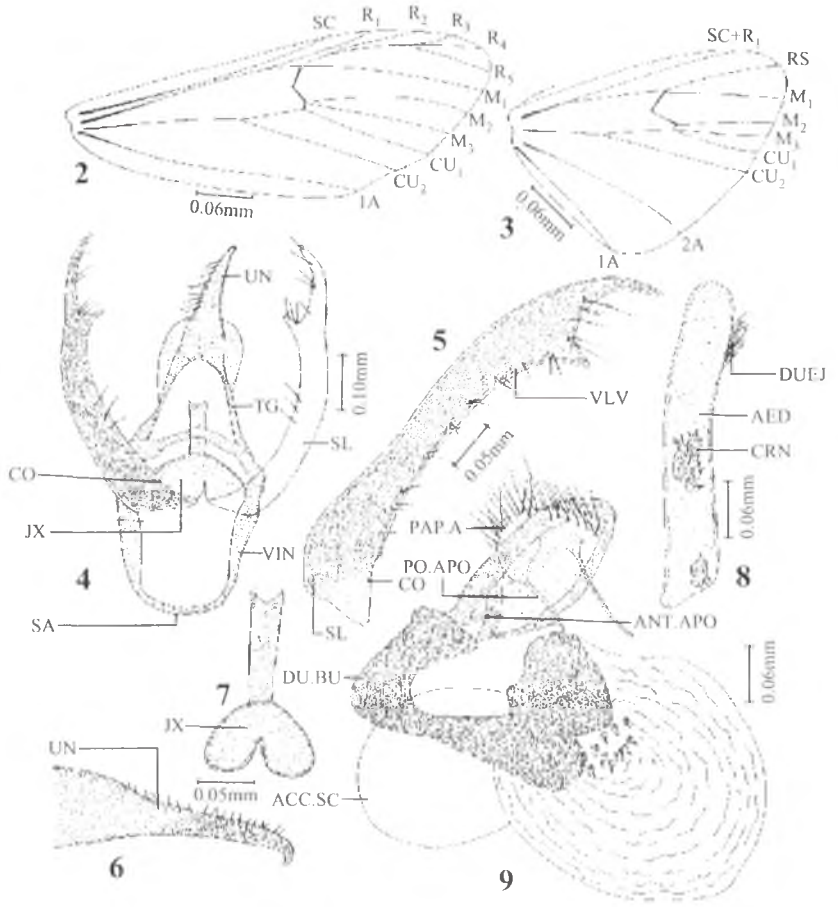
Genus *Creatonotos* Hübner

Hübner, 1827, Verz. Bekar. Sch. 1827:162

Type species: *Creatonotos interruptus* Linnaeus(= *gangis* Linnaeus)

Key to the species of genus Creatonotos Hübner

1. Forewing without costal fascia; a fascia below cell from lower angle of cell to vein M_1, female genitalia with cervix bursae having claw like spines ... *interruptus* (Linnaeus). ... Forewing with white costal fascia; no other fascia present except costal fascia; female genitalia without cervix bursae and claw like spines... 2.
2. Forewing with veins $R_2 - R_5$ stalked from just before upper angle of cell; hindwing with vein M_3 arising from lower angle of cell, Cu_1 from before lower angle of cell; male genitalia with uncus having blunt tip; inner projection of valva short, thorn like; vinculum and saccus broad; acrotergite less developed; aedeagus with vesica bear two patches of spines; female genitalia with anterior apophyses shorter than posterior apophyses... *transiens*(Walker)... Forewing with veins $R_2 - M_1$ stalked from upper angle of cell; hindwing with veins M_3 and Cu_1 from lower angle; male genitalia with uncus having pointed tip; inner projection of valva prominent; vinculum and saccus narrow; acrotergite more developed; aedeagus with vesica having three scobinate patches, a patch at distal end with three prominent spines; female genitalia with anterior apophyses shorter than half length of posterior apophyses... *ananthakrishanani* n.sp.

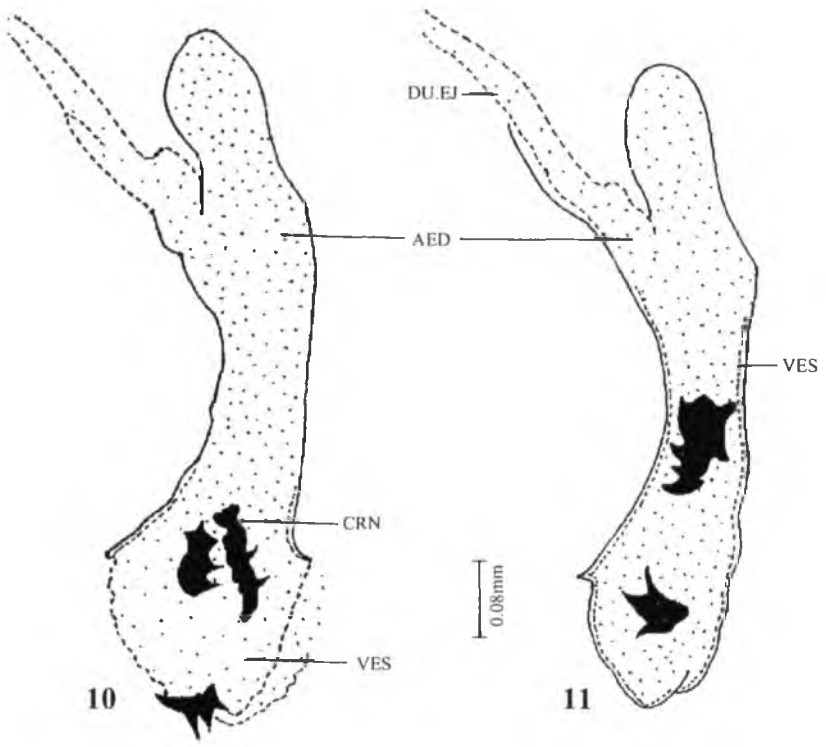


FIGURES 2–9. 2: Forewing of *Creatonotos ananthakrishanani* n. sp. 3: Hindwing of *Creatonotos ananthakrishanani* n.sp. 4–8: Male genitalia of *Creatonotos ananthakrishanani* n.sp. 9: Female genitalia of *Creatonotos ananthakrishanani* n.sp.

Creatonotos ananthakrishanani n.sp.

Head with vertex and frons furnished with white scales. Antenna simple, ciliated; scape studded with black scales; flagellum covered with black and white scales. Eyes black. Labial palpus minute, porrect, just reaching lower level of frons; segments clothed with black scales, underside densely fringed with fuscous scales.

Thorax, collar and tegula covered with white scales; pectus dark fuscous. Forewing with ground colour fuscous brown in male; fuscous white in female; a white costal fascia; black spots in and just beyond each angle of cell; inner margin fringed with



FIGURES 10– 11. 10: Aedeagus of *Creatonotos ananthakrishanani* n. sp. (vesica everted). 11: Aedeagus of *Creatonotos ananthakrishanani* n.sp. (vesica not everted).

white hairs; fringe dark fuscous; in female, a submarginal spot on vein 1 A; veins $R_2 - R_5$ stalked from upper angle of cell. M_2 and M_3 arising from lower angle of cell; Cu_1 from before lower angle of cell; Cu_2 arising beyond two-thirds of cell. Hindwing with ground colour dark fuscous in male, fuscous white in female, fringe white; in female, submarginal series of black spots on veins Rs, M_2, Cu_2 and on, 2 A, point line spot on vein upper angle; vein Sc + R_1 arising just before middle of cell; Rs and M_1 from upper angle; M_2 from above lower angle; M_3 and Cu_1 from lower angle; Cu_2 from middle of cell. Legs dressed with black scales, fore coxae stripped with orange scales; femora orange above, underside fuscous; fore tibia and tarsi streaked with white; hind tibia with one pair of minute spurs, both of equal length.

Abdomen clothed with orange scales, underside with fuscous scales; a dorsal black spot on second segment; lateral, sublateral and ventral series of black spots. To examine genitalic features, the male and female genitalia have been dissected out. For doing so, the entire abdomen was detached from insect body, as cutting of

last few segments often damages the constituent parts of male and female genitalia (Robinson, 1976). The detached abdomen was put in 10 percent KOH for overnight to soften the chitin and to dissolve away the muscles and other unwanted parts. The potashed material was washed in distilled water and residual traces of KOH were later removed by putting it in 1 percent glacial acetic acid. The abdomen was dissected in 50 percent alcohol for taking out the genitalia. Aedeagus is separated from male genitalia by carefully keeping juxta and transtilla intact. After proper dehydration, the genitalic structures were cleared in clove oil and preserved in homeopathic vials containing a mixture of alcohol and glycerine in ratio 8 : 2. The distention of the vesica of the male's aedeagus is helpful for the examination of armature including cornuti and scobination. This is achieved by inserting the hypodermic needle into the base of aedeagus via the entry hole of ductus ejaculatorius and inflating the vesica with alcohol. The diagrams of the genitalia were drawn with the help of a graph eye-piece fitted in zoom stereo binocular on graph papers. Minute structures and setae were given accurate shapes with the help of a microscope.

Male genitalia with uncus moderately long, swollen and rounded at base, then narrow and slender, with both walls slightly convex at distal end, tip hook-like, curved, acrotergite covering basal rounded part of uncus; tegumen long and narrow, inverted V-shaped; vinculum less sclerotized than tegumen; saccus reduced. Valva simple, long and narrow, strongly sclerotized; costa and sacculus poorly defined; distal half bifurcated into blunt processes, one short and another long; juxta large and unique; transtilla well developed. Aedeagus of moderate size with anterior end rounded, both of its walls slightly scelrotized; vesica armed with three scobinate patches, one such patch at a distal end bearing three prominent sclerotized spines. Female genitalia with corpus bursae rounded, bearing circular and slightly sclerotized regular folds; sigum absent, scattered, strongly sclerotized spines present; membranous accessory sac present; ductus bursae sigmoid and highly sclerotized; anterior apophyses very short; posterior apophyses with their apices blunt; papilla analis broad and rounded, setosed with long and fine setae.

Wing Expense(Half) : Male : 22 mm
: Female : 28 mm

Material Examined

Holotype : Uttar Pradesh: Mussorrie, 11.06.94, 1♂.

Allotype : Uttar Pradesh: Mussorrie, 11.06.94, 1♀.

Paratype : Himachal Pradesh: Bhanjuradu, 19.06.93, 1 ♂; 20.06.93,2 ♂♂; Nauni, 01.08.94, 1 ♂, 02.08.94, 1 ♂. Uttar Pradesh: Chakrata, 15.06.94, 1 ♂; Mussorrie, 06.06.93, 1 ♂; 11.06.94, 2 ♂♂; Ranikhet, 02.07.94, 1 ♀. (Coll.Amritpal Singh)

Remarks

The present new species completely conform to the characterization of genus *Creatonotos* Hübner and is closely allied to *C. transiens*(Walker), so far, as the ornamentation of head, labial palpi, wings and legs is concerned. However, it differs from *tran-*

siens with respect to many characters such as hindwing with veins M_3 and Cu_1 arising from lower angle of cell, whereas in *transiens*, Cu_1 arising from before lower angle of cell; abdomen having only one dorsal black spot in new species, whereas the abdomen of *transiens* (Walker) is having a series of dorsal black spots. In present species, male genitalia with uncus having pointed tip, vesica with three scobinate patches, valva with outer projection much longer and juxta also varies in shape, acrotergite more developed and female genitalia with globular corpus bursae, anterior apophyses much shorter in new species as compared to *transiens*(Walker).

Etymology : The new species has been named after the name of an eminent Indian Entomologist, Dr. T. N. Ananthakrishnan.

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ABBREVIATIONS

1A	:	First anal vein	PO.APO	:	Posterior apophyses
2A	:	Second anal vein	R_1	:	First radial vein
ACC.SC	:	Accessory sac	R_2	:	Second radial vein
AED	:	Aedeagus	R_3	:	Third radial vein
ANT.APO	:	Anterior apophyses	R_4	:	Fourth radial vein
CO	:	Costa	R_5	:	Fifth radial vein
CRN	:	Cornuti	RS	:	Radial Sector
CRP.BU	:	Corpus Bursae	SA	:	Saccus
CU_1	:	First cubital vein	SC	:	Subcosta
CU_2	:	Second cubital vein	$SC+R_1$	:	Stalk of $SC+R_1$
DU.BU	:	Ductus Bursae	SL	:	Sacculus
F	:	Frenulum	TG	:	Tegumen
JX	:	Juxta	TRA	:	Transtilla
M_1	:	First median vein	UN	:	Uncus
M_2	:	Second median vein	VES	:	Vesica
M_3	:	Third median vein	VIN	:	Vinculum
PAP.A	:	Papilla Analis	VLV	:	Valva

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Quantitative Intake and Utilization of Cauliflower Leaves by Cabbage White Butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae)

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ABSTRACT: *P. brassicae* larva consumed on an average 3614.2 mg cauliflower leaves (equivalent to 2414.5 mg dry matter) during its entire larval period, of which 85.8% was consumed during the last instar. Maximum growth in terms of gain in weight (289.6 mg) as well as growth rate (0.32) was recorded in the last instar. Approximate digestibility (AD) and efficiency of conversion of ingested food (ECI) declined progressively in the five successive instars to be lowest in the fifth instar whereas efficiency of conversion of digested food (ECD) increased successively to be highest in the last instar. Fresh weight consumption index (CI), AD, ECI and ECD for total larval period were 2.92, 16.33%, 10.17% and 62.25% respectively.

KEYWORDS: Quantitative intake, utilization, *Pieris brassicae*

INTRODUCTION

Quantitative consumption of food by an insect is its total response to food as a whole - its nutrients, water contents and other physical as well as chemical components. Insect nutritional studies yield important information regarding its energy relationship with other biotic components in the ecosystem, and also can contribute for the proper management of the insect species. The present study report is an attempt to quantify the food eaten by *Pieris brassicae* - an important pest of crucifers and other plants, and to understand how it is utilized for the development of the insect.

MATERIALS AND METHODS

Newly hatched larvae of cabbage white butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae) were kept in groups of 20 each in petri dishes (15 replicates). When these reached fourth instar stage, they were segregated and housed singly for further studies. The larvae were provided with a known quantity of food (cauliflower leaves) daily

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TABLE 1. Consumption of cauliflower foliage and faecal matter produced by *P. brassicae*

Instar	Food consumption/larva		Faecal matter/larva		Weight gain/larva
	Fresh food (mg)	Dry matter (mg)	Wet weight (mg)	Dry weight (mg)	(mg)
I	4.3	3.4	2.1	1.3	1.1
II	20.5	16.7	9.5	5.9	6.1
III	72.9	47.7	40.4	28.5	10.9
IV	413.6	271.7	318.3	178.4	59.8
V	3102.9	2075.0	2653.5	1639.9	289.6
Total larval period	3614.2	2414.5	3023.8	1854.0	367.5

at noon till pupation. Daily record of the weight of larva, food offered, food left uneaten and excrement produced was maintained. Control was run concurrently by keeping cauliflower leaves in petri dishes and re-weighing them after 24 hours. The food uneaten, the faecal matter and the control leaves were then dried to constant weight at 60°C in an oven. Various nutritional indices, i.e. consumption index (CI), approximate digestibility (AD), growth rate (GR), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were calculated as per procedure/method given by Waldbauer (1968) on fresh weight basis by taking into consideration the fresh weight of the food (after compensting the natural loss of moisture from it) and the insect as well as on dry weight - fresh weight basis by taking into consideration dry weight of the food and/or faecal matter and the fresh weight of the insect.

RESULTS AND DISCUSSION

The present studies revealed that the *Pieris brassicae* larva consumed, on an average, 4.3, 20.5, 72.9, 413.6 and 3102.9 mg of fresh cauliflower leaves respectively in the five successive instars, thereby consuming a total amount of 3614.2 mg leaves during its entire larval life span. In terms of dry matter, the larva consumed 2414.5 mg in all with the values in the successive five instars being 3.4, 16.7, 47.7, 271.7 and 2075.0 mg respectively (Table 1).

It was observed that the *P. brassicae* caterpillars fed gregariously in the first three instars by scrapping and biting holes in the leaves whereas the later two instars scattered and nibbled at the leaf margins, eating the leaf voraciously leaving behind only the hard mid-rib and veins intact. This change in the mode of feeding from scrapping to biting can be related to the morphology of the mouth parts. Since the maxillae and mandibles get modified in the later instars, these can consume leaves by biting whereas the earlier instars mainly rely on scrapping. Also as the larva grows and enlarges, its nutritional need increases which cannot be fulfilled by scrapping

TABLE 2. Daily consumption of cauliflower leaves by *P. brassicae* larvae

Instar	Day	Average weight of larva	Food consumption			
			Fresh food		Dry matter	
			Per larva	Per gram body weight	Per larva	Per gram body weight
		(mg)	(mg)	(g)	(mg)	(g)
I	1	1.40	2.90	2.07	2.10	1.50
	2	2.50	1.40	0.56	1.30	0.52
II	3	5.10	6.70	1.31	5.40	1.06
	4	8.60	9.50	1.10	8.00	0.93
	5	11.20	4.30	0.38	3.30	0.29
III	6	19.50	49.30	2.53	29.60	1.52
	7	30.40	23.60	0.78	18.10	0.60
IV	8	58.40	128.40	2.20	99.80	1.71
	9	92.20	193.10	2.09	113.40	1.23
	10	118.20	92.10	0.78	58.50	0.49
V	11	150.50	1019.10	6.77	673.30	4.47
	12	301.60	1402.30	4.65	1001.10	3.30
	13	440.10	681.50	1.55	400.60	0.91

of the leaf lamina alone. Trichilo and Mack (1989), Sood *et al.* (1993) and others have reported a high correlation between food consumption and mean larval weight in insects. Consumption of food varies with the metabolic need and state of the insect, and during the present studies, it was observed that the food consumption decreased towards the end of each larval instar even when calculated in terms of food eaten per gram body weight (Table 2).

The studies also clearly indicated that the food consumption per larva as well as per gram body weight increased many folds in the last instar which alone accounted for 85.8% of total consumption during the entire larval period (Table 2), and it conforms to the observations of Mukerji and Guppy (1970); Smith *et al.* (1986); Trichilo and Mack (1989) and Sood *et al.* (1993). King (1981) recorded that virtually half of the feeding in soyabean looper occurred during last two days of larval growth and *P. brassicae* also consumed 57.7% of total food during the last two days of feeding as observed presently. Boldt *et al.* (1975) earlier recorded that 98% of consumption in soybean looper took place in the last three instars.

Present studies revealed that the consumption index calculated on fresh weight basis or on dry weight -fresh weight basis was highest in the fifth instar (Table 3). Also the maximum gain in weight was achieved during the last instar (Table 1). The growth rate was also somewhat higher in the fifth instar. Higher GR indicates higher utilization of ingested food for growth. GR depends upon the nutritional status of the insect (Krishnaswami *et al.*, 1971). Both GR and weight gain in *P. brassicae* indicated that the maximum utilization of the food for tissue synthesis took place in the fifth instar.

TABLE 3. Nutritional indices and growth rate in *P. brassicae* on cauliflower foliage

Instar	GR	Fresh weight basis				Dry weight - fresh weight basis			
		CI	AD(%)	ECI(%)	ECD(%)	CI	AD(%)	ECI(%)	ECD(%)
I	0.28	1.10	51.16	25.58	50.00	0.87	61.76	32.35	52.38
II	0.24	0.82	53.66	29.76	55.45	0.67	64.67	36.53	56.48
III	0.22	1.46	44.58	14.95	33.54	0.96	40.25	22.85	56.77
IV	0.24	1.54	23.04	14.46	62.75	1.01	34.34	20.01	64.09
V	0.32	3.48	14.48	9.33	64.44	2.33	20.97	13.96	66.56
Total larval period	0.23	2.92	16.33	10.17	62.25	1.95	23.21	15.22	65.57

AD was maximum in the first instar and minimum in the fifth instar, ostensibly because the food ingested by fifth instar larvae contained non-digestible cellulosic component in much higher proportion and quantity. This resulted in the production of increased excrement, i.e. 2653.5 mg by wet weight (equivalent to 1639.9 mg dry weight) in the fifth instar (Table 1). AD on fresh weight basis provides better information as water is of utmost importance for insect growth because the efficiency of diet utilization increases with increased water contents in the food (Reese and Beck, 1978; Reynolds *et al.*, 1985).

Fresh weight efficiency of conversion of ingested food in *P. brassicae* decreased from 23.26% in the first to 9.22% in the last instar; the corresponding values on dry weight - fresh weight basis being 32.35 and 13.96% respectively (Table 3). ECD on the other hand increased progressively in the successive instars and was maximum in the last instar. For total larval period, the ECI and ECD were 10.17 and 62.25% respectively on fresh weight basis and 15.22 and 65.57% respectively on dry weight - fresh weight basis. ECI tends to decrease with the increase in age in most of the insects, partly due to a concomitant decline in AD. An increase in ECD with declining AD and ECI can be attributed to the fact that insect utilizes more food for assimilation in the tissue in the later stages as manifested by increase in the weight of the insect.

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Description of two new species of *montium* subgroup of *Drosophila* (Diptera: Drosophilidae) from South India

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ABSTRACT: This study describes two new species, *D. palniensis* collected from Palni hills and *D. neolacteicornis*, from Nilgiri hills, India. Their taxonomic status and relationships are discussed.

KEYWORDS: *D. palniensis*, *D. neolacteicornis*, Palni hills, Nilgiri hills

INTRODUCTION

The Indian sub-continent with its tropical climate and varied physiographic conditions offers a variety of insect fauna. The genus *Drosophila* is one such group of insects that has about 2240 biologically valid species (Wheeler, 1986).

The study of Indian *Drosophilids* was started by Bezzi (Sturtevant, 1921). Much of our present knowledge on *Drosophila* fauna has been acquired only after 1964. The species collected in the Indian sub-continent up to 1974 is enlisted by Gupta (1974). After 1974 about 40 new species have been added to the list from India including Andaman Islands. Inspite of the above information, many areas of the Indian sub-continent still remain unexplored. Hence the present survey was undertaken to explore new species of *Drosophila*.

MATERIALS AND METHODS

Collections of *Drosophila* from Palni and Nilgiri hills, Tamil Nadu were made in hill ranges using net sweeping as well as bottle trapping methods from 11 different altitudes (350, 475, 800, 950, 1050, 1150, 1450, 1650, 1750, 1800, and 2300 m above sea level). This collection resulted in the finding of two new species; one from Palni hills and another from Nilgiri hills which are described here.

RESULTS AND DISCUSSION

The description of the two species is as follows.

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TABLE 1. Wing Indices of *D. palniensis*

Sex	Costal index	4 C index	4 V index	5 V index
Male	2.01	1.00	1.62	0.56
Female	2.14	1.07	1.69	1.62

Drosophila palniensis, Hegde and Shakunthala sp. nov.

Males and Females: Brownish yellow, females are larger than males.

Head: Arista with 5/3 branches excluding terminal fork in females. In male, arista with 4/3 branches including terminal fork.

Antenna: Yellow, palpi with bristles and another with thin bristle; carina narrow, vibrissae with two anterior prominent bristles and two thin small posterior bristles. Anterior and middle orbitals are of same size but posterior orbitals longer than anterior and middle. Anterior orbitals proclinate whereas posterior orbitals reclinate. Anterior verticals large and directed inwards; posterior verticals directed outwards. Ocellar triangle with a pair of bristles. Eyes are red.

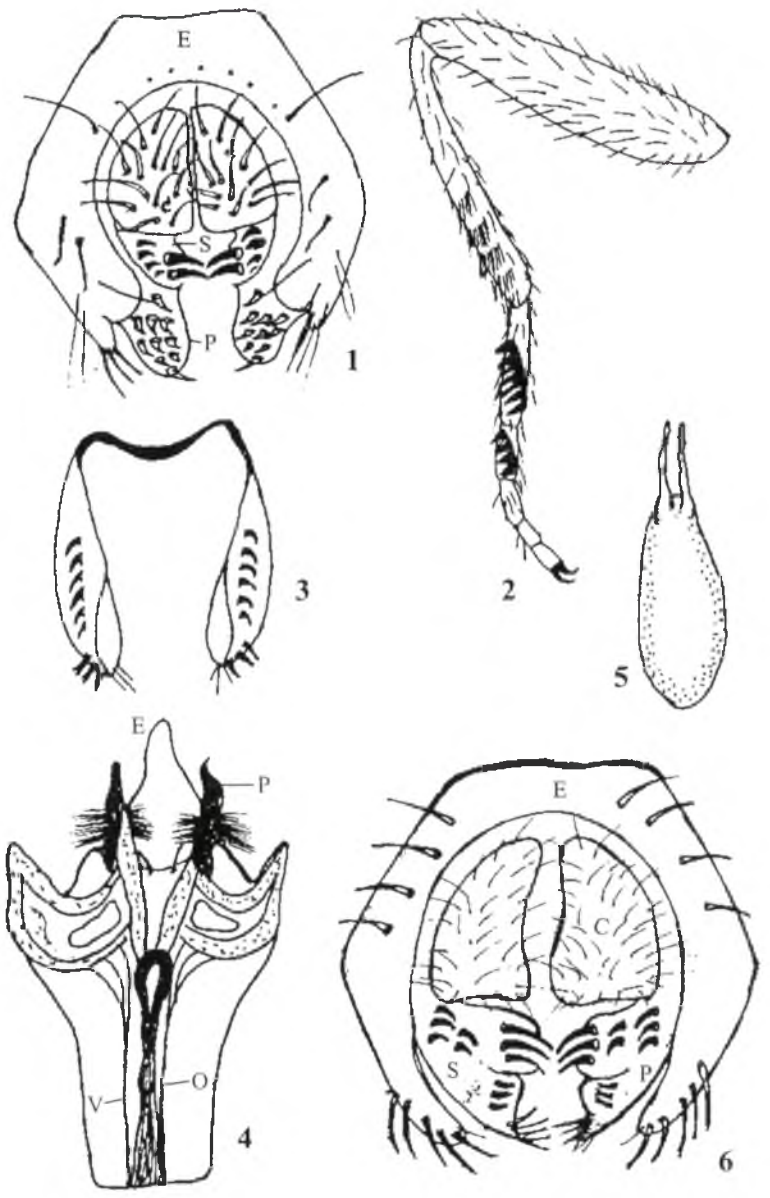
Thorax: Brownish yellow. Acrostichal hairs in eight rows. Anterior dorsocentrals are shorter than posterior dorsocentrals, posterior dorsocentrals convergent. Anterior scutellars convergent and posterior scutellar convergent and crossed. Both anterior and posterior scutellars are of equal length. Both anterior and posterior alars are equal in length and directed downwards. There are 5 sternopleural bristles out of which 4 are smaller and fifth one is dark and prominent. Halteres transluscent.

Wings: Smoky and hyaline. Wing length; male: 2.4 mm: female 2.45 mm. The wing indices are calculated following the formula of Okada (1956) and presented in Table 1.

Legs (Fig. 2): First tarsal segment of the forelegs in male carries two sets of sexcombs. Proximal set with 7 short and thick teeth and the distal set with 4 short and thick teeth. In femur a row of thick bristles present in the fore leg of male. This is absent in the females. Preapicals on tibia.

Abdomen: Bright yellow, tergites darkely pigmented at apical margins. Pigmentation broader on the mid dorsal portion of the tergites and narrowed laterally, Further intensity of pigmentation is more in male. Posterior part of males abdomen is shiny black.

Egg (Fig. 5): Egg with two filaments. They are short and pointed at the tip.



FIGURES 1–6. 1: Periphallic organ: E-Epandrium; C-Anal cercus; P-Primary surstylus; S-Secondary surstylus; 2: Sexcomb: 3: Egg guide; 4: Phallic organ: E-Aedeagus; P-Posterior gonapophyses; A-Anterior gonapophyses; O-Apodeme; V-Ventral fragma; 5: Egg. 6: Periphallic organ; E-Epandrium; C-Anal cercus; P-Primary surstylus; S-Secondary surstylus;

Internal Structures: Ovary with eight ovarioles, testis yellow with 3 coils.

Egg guide (Fig. 3): Brown with 6–9 teeth.

Phalic organ (Fig. 4): Yellowish brown, aedeagus large curved dorsally with hairy sensilla, basal apodeme projecting beyond fragma. Anterior gonopophysis are short and triangular. Posterior gonopophysis long slender and non serrate.

Periphallic organ (Fig. 1): Epandrium broad dorsally and laterally, toe long and round with 6 bristles, primary and secondary surstylus present. Primary surstylus with 11 bristles arranged in 3–4 rows. Secondary surstylus is continuous with cerci and carries two large, black bristles directed downwards. In addition to that a row of 3 small bristles arranged laterally with 18–20 bristles.

Holotype: 2 ♂♂, India, Tamil Nadu Palni hills, 9 vii 1997. coll. S. N. Hegde, V. Vasudev, M. S. Krishna, V. Shakunthala and K. Raviram.

Allotype: 2 ♀♀ same as above,

Paratype: 2 ♂♂ and 2 ♀♀, India, Tamil Nadu, Palni hills. coll. S. N. Hegde and others.

Relationships: The species under description belongs to subgenus *Sophophora* and *melanogaster* species group in having shiny black abdomen in males, long coiled ventral receptacle, coiled testis, sexcomb, eggs with two filaments etc., The males have yellowish abdominal tergites with distinct apical bands, prominent sexcomb in two sets, genital arch not constricted above, secondary surstylus fused to analcercus two prominent curved teeth on secondary surstylus, aedeagus hirsute, anterior parameres large. Therefore this species has been included under *montium* subgroup.

The species resembles *D. serrata* (Bock and Wheeler, 1972) in having two prominent large teeth on the secondary surstylus, but differs from it significantly in sex comb pattern. The new species has prominent sexcomb with seven and four teeth each, which are not fused. Therefore the species is considered as new and named as *D. palniensis*. This new species belongs to *montium* subgroup and it is related to *Drosophila serrata*.

Drosophila neolacteicornis, Hegde and Krishna sp.nov

Males and females: Light grey color, females are larger than males.

Head: Arista with 4/2 branches excluding terminal fork. Antenna yellowish grey, palpi with bristles, carina narrow, vibrissae with two anterior and posterior prominent bristles. Anterior and middle orbitals are small and reclinate. The posterior orbitals are longer than the middle and shorter than anterior. Anterior verticals are large

TABLE 2. Wing indices of *D. neolacteicornis*

Sex	Costal index	4 C index	4 V index	5 V index
Male	1.41	0.73	1.14	0.38
Female	1.48	0.82	1.24	0.46

and directed inwards whereas posterior verticals are convergent and crossed. Ocellar triangle with a pair of dark bristles. Eyes dark red.

Thorax: Greyish yellow, Achrostichals are in eight rows, anterior dorsocentrals shorter than posterior. Anterior scutellar bristles convergent whereas the posterior scutellars convergent and crossed. Anterior scutellars are smaller than posterior. The anterior and posterior alars are equal in length and directed downwards. There are five sternopleural bristles out of which three are small, two are prominent and dark. Halteres transluscent.

Wing: Transparent, smoky and hyaline. Wing length, male: 1.19 mm, female: 1.68 mm. The wing indices are calculated following the formula of Okada (1956) and presented in Table 2.

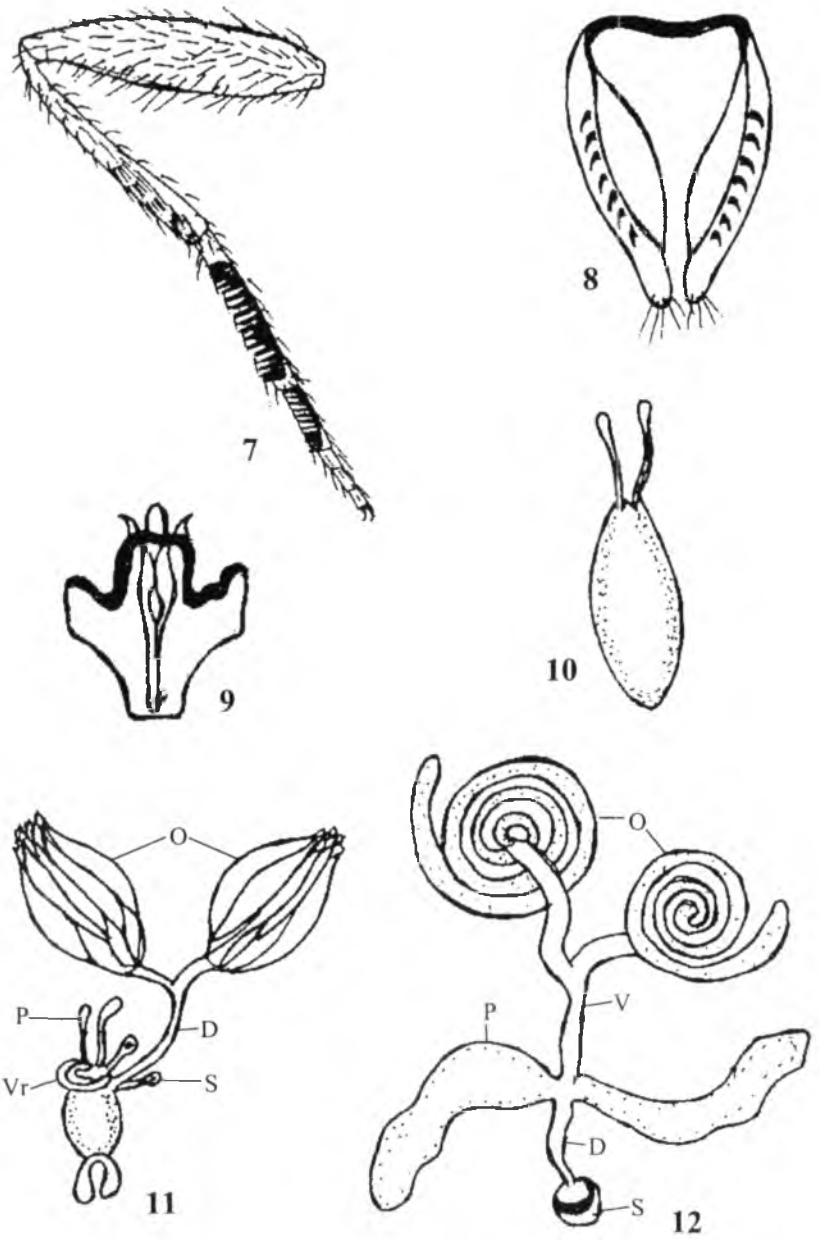
Legs: The first tarsal segment in males carries 2 sets of sex comb (Fig. 7). Proximal set with 17–22 teeth and the distal set with 12–14 teeth, few anterior teeth of the proximal set and few posterior teeth of distal set are united. Preapicals on all tibia.

Abdomen: Bright yellow, tergites darkly pigmented at posterior margins when compared to anterior margins. Further the intensity of pigmentation is more in male when compared to female. Posterior abdomen of males are darkly pigmented.

Pupa: Yellow with eight spiracular filaments, at the posterior end there are five pairs of projections of which two are lateral, two are dorsal and one pair is ventral in position.

Egg (Fig. 10): Egg with two filaments. They are slender and slightly flatened apicaly.

Periphallic organs (Fig. 6): Epandrium broad, apically narrow and concave, heel constricted, toe rounded with 6–7 bristles and curved inwards. Both primary and secondary surstylus are present, primary surstylus with a set of 3 short teeth arranged in a row and 8–10 irregularly arranged teeth at the proximal end. Secondary surstylus continuous with analcercus. A set of three dark teeth are present on the inner margin of the secondary surstylus of which posterior one is short. In addition there are also two additional sets of teeth are found. The median set has two teeth and outer one has three.



FIGURES 7–12. 7: Sex comb; 8: Egg guide; 9: Phallic organ: E-Aedeagus; P-Posterior gonapophyses; A-Anterior gonaphophyses; O-Apodeme; V-Ventral fragma; 10: Egg: 11: Female reproductive system: D-Oviduct; O-Ovary; P-Paragonia; S-Spermathecae; Vr-Ventral receptacle; 12: Male reproductive system; D-Anterior ejaculatory duct; P-Acessory gland; S-Ejaculatory bulb; T-Testis; V-Vas deferens.

Egg guide (Fig. 8): Brown with 6–8 teeth.

Phallic organ (Fig. 9): Aedeagus brownish yellow, long and broad basally, Anterior gonopophysis are small with few apical sensilla. Posterior gonopophysis are large with a chitinous spine which is directed posteriorly. Ejaculatory apodeme long, ventral fragma broad dorsally and laterally.

Internal structure (Fig. 11 & 12): Ovary with 8–10 ovarioles. Ventral receptacle is tightly coiled, spermatheca small. Testis yellow with 4–5 coils. paragonia relatively smaller than testis.

Holotype: 2 ♂♂, India, Tamil Nadu, Nilgiri hills, 14. xi 1996. coll. L. Siddaveeregowda, H. A. Ranganath, S. N. Hegde, S. R. Ramesh and N. B. Ramachandra.

Allotype: 2 ♀♀: same as above

Paratype: 5 ♂♂ and 2 ♀♀, India, Tamil Nadu, Nilgiri hills, coll. L. Siddaveere Gowda, H. A. Ranganath, S. N. Hegde, S. R. Ramesh and N. B. Ramachandra.

Relationships: Most of the character pointed out in *Drosophila palniensis* for *melanogaster* species group of *Sophophora* and *montium* subgroup were also seen in this species. This species resembles *D. lacteicornis* (Okada, 1965) in having three curved teeth on the secondary surstylus of which lower one is smaller than the other two, but it is significantly different from *D. lacteicornis* in having lateral row of three pointed teeth on primary surstylus and on the secondary surstylus besides three curved black teeth, two pointed small teeth are found medianly. Hence this species is considered as new and named as *Drosophila neolacteicornis*. This new species seems to be related to *D. lacteicornis*.

Key for identification: In view of the description of two new species of montium subgroup, the existing key for identification of the new species (Gupta, 1974) may be modified as follows.

1. Dark bands of abdominal tergites when present not medianly interrupted. Eggs with two filaments (*Sophophora*) ..... 2.
2. Yellowish abdominal tergites with distinct apical bands, prominent sexcomb in two sets, genital arch not constricted above, secondary surstylus fused to analcercus, aedeagus hirsute, anterior parameres large (*montium* subgroup) ... 3.

3a. Two prominent large teeth on the secondary syrstylus ..... 4.

b. Three curved teeth on the secondary surstylus of which lower one is smaller than the other two ..... 5.

4a. Prominent sexcomb with seven and four teeth each ..... *D. palniensis*

b. Sexcomb with teeth on upper portion of metatarsus and on entire second tarsal segment, fine, densely packed, contiguous ..... *D. serrata*

5a. Lateral row of three pointed teeth on primary surstylus and on the secondary surstylus besides three curved black teeth, two pointed small teeth are found medianly ..... *D. neolacteicornis*

b. Total five teeth on the secondary surstylus of which three are lateral and curved while remaining two are smaller and vertical in position ..... *D. lacteicornis*.

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Morphometric Determination of Larval instars of *Ailanthus* defoliator, *Eligma narcissus indica* Roth. (Lepidoptera: Noctuidae)

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ABSTRACT: Studies on morphometric determination of Larval Instars of *Eligma narcissus indica* in Kerala is reported here. There are six larval instars, distinguishable by size and specific morphological features. The ratio of head capsule width of successive instars is 1.5 except II for which it is 1.4. The ratio of faecal pellet width of successive instars is also more or less constant ranging between 1.5 and 1.9. The relationship between larval instars and log width of head capsule/faecal pellet is strainght lines. Multiple regression analysis of morphometric data show significant relationship between body size and head capsule width/faecal width in the successive larval instars.

KEYWORDS: *Ailanthus* defolicator, *Eligma narcissus indica* Roth, larval instar determination.

INTRODUCTION

Ailanthus triphysa is a fast growing soft wood tree of considerable economic importance in Kerala for match, packing case and paper pulp industries. The major monophagous pest of *Ailanthus* in Kerala, as elsewhere in India, is *Eligma narcissus indica* Roth. (Lepidoptera: Noctuidae) (Varma, 1986). Fragmentary information on the biology of this pest has been given by Bhasin and Roonwal (1954); Chatterjee and Sen-Sarma (1968); Chatterjee *et al.* (1969) and Joseph and Karnavar (1991, 1993). Though the various life - history stages have been briefly described by Roonwal (1982), very little attention has been focussed on morphometric determination and visual identification of different larval instars of this serious defoliator insect in forest plantations, homesteads and nurseries of *A. triphysa* in Kerala. In the present paper an attempt has been made to bridge this lacuna.

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MATERIALS AND METHODS

Eggs of *Eligma narcissus indica* were collected from the field and all the subsequent stages were reared in the laboratory. The neonate larvae were transferred to rearing glass bottles (10 × 18 cm.) and were maintained on leaves of *A. triphysa* at laboratory temperature and humidity and 12 : 12 LD photoperiod approximately. The later instars (IV, V and VI) were reared in cylinderical wire-mesh cages (26 × 45 cm.) in order to provide the growing larvae sufficient space. The pupae were collected everyday and kept for adult emergence in separate cages and the moths were fed on 10% sucrose solution.

A total of 30 egg clusters collected during different periods between July - December were used for the present study. The neonate larvae were observed for their moult. The observation was made for a duration of one year covering peak period (between July and November) and lean period (between December and June) on ten batches, each containing 8–10 newly hatched larvae. Measurements of the whole animals were taken on a millimeter scale while earlier instar larvae, head capsules and faecal pellets were measured under a binocular dissection microscope fitted with ocular micrometer. Gravimetric study was made using an electronic digital balance.

Using the morphometric data of larva, the multiple regression equation (Snedecor and Cochran, 1967) defining the relationship of length ($X1$) and width ($X2$) of the body with the width of head capsule ($Y1$)/faecal pellets in the successive larval instars ($Y2$) was obtained. In all the case, the observed fitted regression was tested using the 'F' test. Linear regression of head capsule width on faecal pellet width was also tried.

RESULTS AND DISCUSSION

There are six larval instars and the larval stage is completed within a period of 21–24 days. The morphometric details of the different larval instars of the insect are shown in Table 1 and Fig. 1. The number of larval instars was determined based on the head capsule width and faecal pellet size. The determination of the larval instars is mostly based on measurements of head capsule width, eventhough the size of the faecal pellets is sometimes taken as a criterion especially in the case of stored - product insects (Sardesai, 1969). Variable number of larval instars ranging from five to eight has been reported in different lepidopterous insect species based on head capsule width (Neal, 1984; Smith, 1984; Goodman *et al.*, 1985; Smith *et al.*, 1986; Santhosh Babu and Prabhu, 1987; Annie John and Muraleedharan, 1989; Gupta *et al.*, 1989). In several other insect species too head capsule width is considered as the criterion in determining the larval instars (De Oliveira and Durand, 1978; Nemjo and Slaff, 1984; Shinkarenko *et al.*, 1986).

Following the multiple regression equation in this insect, width of the head capsule/faecal pellets were computed using the morphometric data, and was found in good agreement with the observed mean widths as reported in *Henosepilachna vigintioctopunctata* (Gupta and Kumar, 1983) and in *Achoea janata* (Annie John, 1991).

TABLE 1. Morphometric details of different larval instars of *Eligma narcissus indica*

Larval instar	number of insect used	duration (days)	final day body length (L) in mm	final day body width (B) in mm	growth ratio between successive instars		weight on final day* (mg)	head capsule width (mm)	RHW**	faecal pellet width (mm)	RFW***
					L/L	B/B					
I	90	2.93 ± 0.19	3.80 ± 0.30	0.68 ± 0.06	1.73	1.83	1.17 ± 0.28	0.41 ± 0.01	–	0.15 ± 0.01	–
II	78	3.05 ± 0.15	6.57 ± 0.55	1.25 ± 0.13	1.69	1.45	4.64 ± 0.76	0.58 ± 0.03	1.4	0.24 ± 0.01	1.6
III	61	3.15 ± 0.23	11.13 ± 0.82	1.81 ± 0.63	1.46	1.22	13.71 ± 1.28	0.87 ± 0.07	1.5	0.36 ± 0.03	1.5
IV	52	3.18 ± 0.31	16.28 ± 1.63	2.21 ± 0.12	1.63	1.82	34.74 ± 6.19	1.29 ± 0.06	1.5	0.56 ± 0.03	1.5
V	48	4.08 ± 0.37	26.58 ± 1.44	4.03 ± 0.21	1.37	1.24	132.68 ± 15.33	1.99 ± 0.08	1.5	0.93 ± 0.09	1.7
VI (feeding larva)	48	4.71 ± 0.59	36.53 ± 1.47	4.99 ± 0.12	0.80	1.25	554.80 ± 57.68	2.99 ± 0.12	1.5	1.78 ± 0.11	1.9
VI (non-feeding spinning larva)	48	0.63 ± 0.18	29.31 ± 1.37	6.24 ± 0.11	0.84	1.07	486.56 ± 47.50	2.99 ± 0.12			
VI (prepupa)	48	1.90 ± 0.16	24.48 ± 1.81	6.67 ± 0.34	0.93	1.08	432.15 ± 35.01	2.99 ± 0.12			

* Number of insect used 10, ** RHW - Ratio of Head capsule width between successive larval instars.,
*** RFW - Ratio of faecal pellet width between successive larval instars.

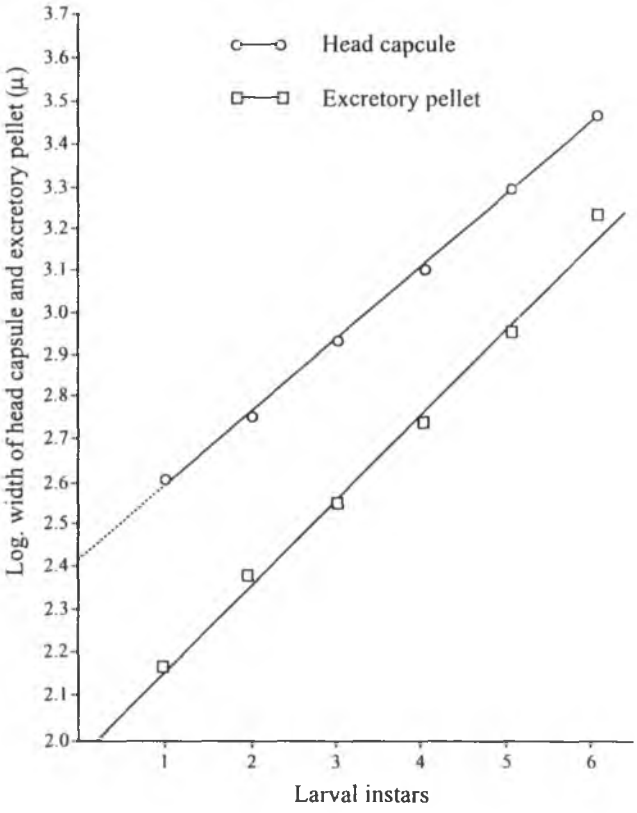
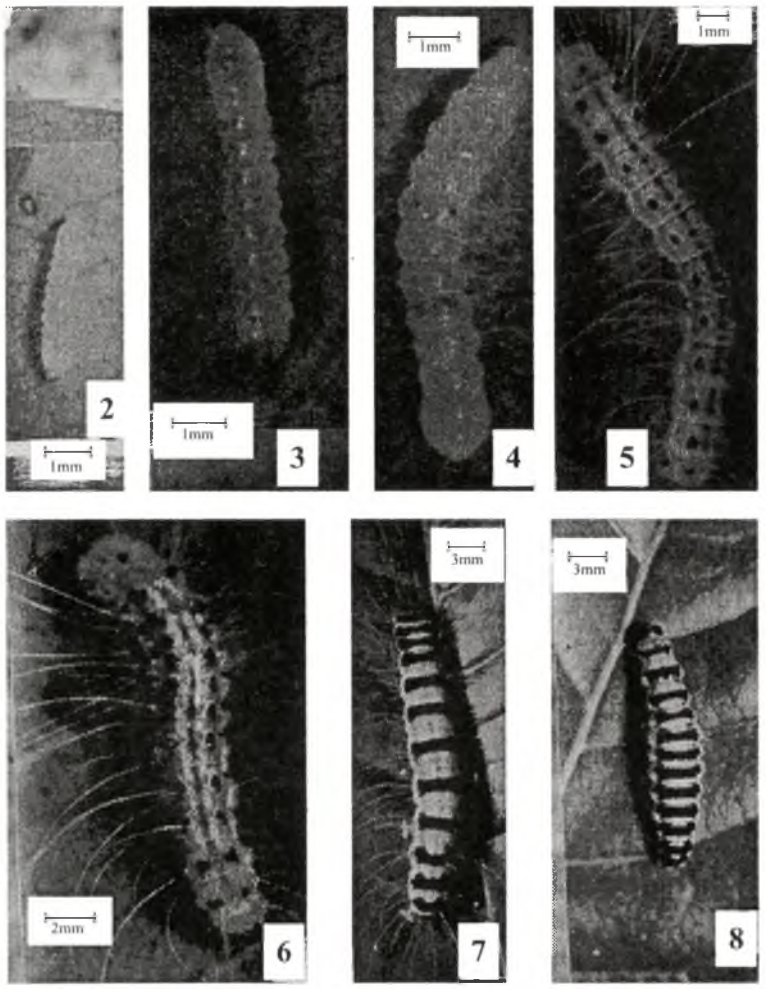


FIGURE 1. Relationship of Head Capsule width and Excretory Pellet width with larval instars in *Eligma narcissus indica*

Dyar's law of linear growth holds that the width of the head capsule of larva increases in its successive stages in a regular geometric progression by a constant ratio of 1.4 (Dyar, 1890; Dyar and Rhinebeck, 1890). The present study revealed that the relationship between larval stages and log of head capsule width in this insect is straight line and ratio of increase in head capsule width was 1.5 for all the larval instars except II for which it was 1.4. On the whole Dyar's rule was found to hold good agreement, but with a slight increase from 1.4 to 1.5 (Table 1 and Fig. 1). It is shown that when there are supernumerary instars due to malnutrition the ratio tends to be lower (Seamans and Woodruff, 1939). The relationship between larval stages and log of faecal pellet width in this insect was also straight line and the ratio showed a proportionate increase in succeeding instars, but the values differ from that of head capsule widths (Table 1 and Fig. 1). However linear regression analysis of head capsule width on faecal pellet width showed a positive correlation as reported by Sardesai (1969) in *Ploida interpunctella.*



FIGURES 2–8. Successive larval instars of *Eligma narscissus indica* 2: I instar; 3: II instar; 4: III instar; 5: IV instar; 6: V instar; 7: VI instar; 8: VI instar (prepupa)

In *Eligma narcissus indica* various larval instars could be visually identified on the basis of colour changes, size differences and specific morphological features after each moult (Fig. 2–8). The duration of larval instars and widths of the head capsule and faecal pellets of the insect are presented in Table 1. The first instar larvae were whitish thread - like with soft setae and without black spots. They emerged by making a hole in the egg shell and were ready to commence feeding irregularly on the lower epidermis of the leaves. During the second instar the larval body became spongy and

the colour slowly changed to greenish yellow. Paired black spots developed dorsally on the anteriormost five abdominal segments and in some cases paired black spots also appeared on the posterior segments too. Third instar larvae were comparatively larger in size with paired black spots dorsally on all segments. In the fourth instar, the paired black spots were prominent whence arise long rigid setae. Unpaired middle black spots also begain to appear dorsally and the larvae started to feed on the entire leaf tissue. In the fifth instar larvae the black spots became much more prominent and enlarged to wart - like structures. The sixth instar larvae became fairly large in size. The larval body was spongy, whitish yellow in colour with dark brown transverse band in each segment dorsally, triangular black mark above the mouth and large paired eye - like spots on the head capsule. Visual identification of larval instars based on morphology after each moult has also been reported in a number of lepidopterous insects (Schabel *et al.*, 1988; Gupta *et al.*, 1989; Misra *et al.*, 1991).

The VI instar larval period included three stages - feeding stage of 4.71 ± 0.60 days, non-feeding spinning stage of 0.63 ± 0.18 day and pre-pupal stage of 1.90 ± 0.16 days (Table 1). At the termination of feeding stage, the larvae moved in search of suitable sites on the stem of the host plants for cocoon construction and subsequent pupation. Regarding the site of cocoon construction, there was no specificity, sometimes cocoon construction and pupation occurred in the nearby plants or even on the walls of the rearing cages. The gravimetric analysis showed that maximum weight attained was during the last period of the last instar before spinning and thereafter body weight decreased as in the case of *Galleria mellonella* (Janda *et al.*, 1966). This decrease could be related to the non-feeding, cocoon formation, emptying of intestine, water loss etc.

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Additional record of insect predators of Coconut lace bug *Stephanitis typica* (Distant) and studies on biology and feeding potential of *Euagoras plagiatus* Burmeister (Heteroptera: Reduviidae)

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ABSTRACT: Search for natural enemies of the lace bug *Stephanitis typica* revealed the presence of *Euagoras plagiatus, Occamus typicus* and *Geocoris flavipes* associated with lace bug colony, which are new record of predators on lace bugs. Biology and predator-potential of *E. plagiatus* were studied on *S. typica, Corcyra cephalonica* and *Oecophylla smaragdina*. Type of prey consumed has significant influence on the life cycle of the predator. Nymphal duration is prolonged when the predator was fed on *S. typica*. Eventhough the fifth instar nymphs consumed more number of lace bugs than the other instars, they failed to moult as adults. But when supplemented with *Corcyra* larvae they moulted as adults. *E. plagiatus* showed a fecundity rate of 263.17 ± 51.59 and 82.67 ± 26.77 eggs, when fed on *C. cephalonica* and *S. typica*, respectively. Combined with the already recorded natural enemies, this predator could effectively check the lace bug population.

KEYWORDS: Lace bug, *Stephanitis typica*, predators, Reduviidae, *Euagoras plagiatus*, Coconut

INTRODUCTION

The lace bug *Stephanitis typica* (Distant) is a pest on coconut palm foliage. As a pest it has a minor role to play but it causes damage to coconut palms as vector of root (wilt) disease (Mathen *et al.*, 1990). Many natural enemies were found associated with the lace bug in the field. Among these, the important predators are *Stethoconus praefectus* D. (Miridae), *Endochus inornatus* Stal. (Reduviidae), *Rhinocoris fuscipes* Fabr. (Reduviidae) and *Ankylopteryx octopunctata octopuntata* F. (Chrysopidae) (Mathen *et al.*, 1967; Mathen and Kurian, 1972; Sathiamma *et al.*, 1998). During our field observations *Euagoras plagiatus* Burmeister and *Occamus typicus* Distant (Heteroptera: Reduviidae) and *Geocoris flavipes* (B.) (Heteroptera: Lygaeidae) were found feeding on *S. typica*, which are not yet reported.

Among the different predators *E. plagiatus* is one of the common reduviid predators

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found on lace bug under field conditions. Detailed studies were taken up on the biology and feeding potential of this predator under laboratory and field conditions to determine its potentiality as a biocontrol agent of *S. typica*.

MATERIALS AND METHODS

Search for natural enemies of *S. typica* was carried out at three locations viz. Ayiromthengu, Krishnapuram and Thottappally in Kerala.

E. plagiatus adults collected from coconut nursery at Krishnapuram were maintained in the laboratory of *Corcyra* larvae at a temperature of 28–33 °C and relative humidity 77 to 93%. Females laid eggs on cotton plugs of test tubes in the laboratory and they were kept separately for hatching and the newly hatched nymphs were then used for studies on the biology and feeding potential. Tests on biology and prey consumption were conducted on three different preys viz. nymphs and adults of *S. typica*, larvae of *Corcyra cephalonica* S. and pupae of *Oecophylla smaragdina* Fabr. Field collected adult lace bugs were released on coconut palms maintained in the field cage and were allowed to multiply. From this culture sufficient number of lace bugs were collected each time for biology and feeding potential studies. *C. cephalonica* culture was maintained in the laboratory on sooji. *O. smaragdina* pupae were collected from field nests and stored in refrigerator and used as prey for the lace bug.

Predation was studied by placing predator stages singly in test tubes (15 cm long and 2.5 cm wide) covered with cotton plugs. Each predator was exposed to 5 nos, each of *Corcyra* larvae and *Oecophylla* pupae and 15 nos. of lace bugs separately at 24 h interval. For the nymphs, predation was monitored through out the instars. Total number of prey killed in each nymphal instar was recorded at 24 h interval. Prey killed by the predator was replaced daily to keep prey density constant through out the experiments. Developmental duration and survival of the predator on each prey was recorded separately every day. Prey consumption by the adult predators were also recorded similarly. Nymphal predation was expressed as the number of prey killed during the instar while for adults as the number of prey killed per day. Fecundity and longevity of the adult predators were also recorded.

Adults emerged from feeding on *Corcyra* larvae and *Oecophylla* pupae were monitored for their feeding potential on lace bugs maintained in caged coconut seedlings. For this study, 24 h old adults were released singly on to the lace bug (50 nos) infested leaves covered with nylon net cages. The number of prey fed by predators was recorded after 24 h of release. Care was taken to maintain a constant number of prey on the leaves.

Effect of different temperatures on the rate of development of *E. plagiatus* on *Corcyra* larvae were also studied. This test was conducted at two different fixed temperatures ie. at 20 °C and 25 °C and is compared with the normal 30 °C. Developmental period of the predator was recorded through out the nymphal stages.

Controls were also maintained for all the experiments.

TABLE 1. Duration of different nympal instars of *Euagoras plagiatus* on different hosts in laboratory. (Mean ± SD)

Nymphal instars	Duration (in days) on		
	*S. typica**	*C. cephalonica**	*O. smaragdina***
I	6.00 ± 0.85	7.00 ± 1.19	6.00 ± 1.00
II	5.92 ± 0.76	7.00 ± 1.41	5.67 ± 1.15
III	7.75 ± 2.00	5.46 ± 1.19	5.33 ± 0.58
IV	10.58 ± 2.43	6.57 ± 1.22	6.00 ± 0.00
V	22.90 ± 6.85	8.67 ± 1.30	9.67 ± 0.58
Total Nymphal Period	53.10 ± 5.88	34.70 ± 6.31	32.67 ± 2.08

* Number of replications - 15
** Number of replications - 5

RESULTS AND DISCUSSION

Euagoras plagiatus

Biology: Adult bugs, of both sexes are dark brown in colour with two conspicuous spines on the thoracic region. Nymphs are yellowish brown in colour. Female lays cylindrical shaped, brown coloured eggs, singly.

E. plagiatus completes its life cycle on *S. typica* in 62.20 ± 0.48 (range 60–63) days. Egg period is completed in 6.83 ± 0.38 (range 6–8) days. Nymphal period is completed in 53.1 ± 5.88 (range 48–58) days covering the first instar nymphal period in 6.00 ± 0.85 (range 6–8); second instar in 5.92 ± 0.76 (range 5–7); third instar 7.75 ± 2.00 (range 5–9); fourth instar 10.58 ± 2.43 (range 8–12) and fifth instar 22.90 ± 6.85 (range 14–28) days (Table 1).

Studies on biology using *C. cephalonica* as the prey revealed that egg to adult period is completed in 41.53 ± 3.98 (range 36–48) days, with an egg period of 6.83 ± 0.38 (range 6–8) and nymphal period of 34.70 ± 6.31 (range 30–38) comprising the first instar period of 7.00 ± 1.19 (range 6–8); second instar 7.00 ± 1.41 (range 6–8); third 5.46 ± 1.19 (range 4–7); fourth 6.57 ± 1.22 (range 5–7) and fifth 8.67 ± 1.30 (range 7–9) days at 30 °C (Table 1). At 25 and 20 °C the nymphal period were completed at 35.00 ± 2.24 and 103.98 ± 7.64 days, respectively, as compared to 34.70 ± 6.31 days at 30 °C (Table 2).

When *O. smaragdina* is used as prey the egg period remains the same as that of *S. typica* and *C. cephalonica*. The nymphal period is completed in 32.67 ± 2.08 (range 30–36) days comprising the first to fifth instar 6.00 ± 1.00, 5.67 ± 1.15, 5.33 ± 0.58, 6.00 ± 0.00 and 9.67 ± 0.58 days, respectively (Table 1).

It was observed that the type of prey consumed had remarkable influence on the life of the predator. Duration of the nymphal instar was longest when the predator was fed on *S. typica*, whereas it was comparatively short 34.70 ± 6.31 and 32.67 ± 2.08, respectively, when fed on *C. cephalonica* and *O. smaragdina* (Table 1). It was also

TABLE 2. Duration of Nymphal instars of *Corcyra* fed *Euagoras plagiatus* (in days) at different temperature (Value Mean ± SD; Number of replications - 15)

Nymphal instars	Temperature		
	20 °C	25 °C	30 °C
I	11.40 ± 0.89	6.00 ± 0.82	7.00 ± 1.19
II	13.25 ± 1.89	6.00 ± 0.00	7.00 ± 1.41
III	12.00 ± 0.82	4.50 ± 0.71	5.46 ± 1.19
IV	28.33 ± 0.58	6.50 ± 0.71	6.57 ± 1.22
V	39.00 ± 3.46	12.00 ± 0.00	8.67 ± 1.30
Total Nymphal days	103.98 ± 7.64	35.00 ± 2.24	34.70 ± 6.31

observed that nymphs of the predator fed only on lace bug failed to moult (even when it was observed up to 14 days) at the fifth instar stage as adults. But these fifth instar nymphs when fed on larvae of *Corcyra* readily moulted and entered the adult stage (Table 1).

Feeding potential The number of *C. cephalonica* larvae, *S. typica* adults and nymphs and *O. smaragdina* pupae consumed by *E. plagiatus* during different instars are given in table 3. During the first instar period *E. plagiatus* fed 15.67 ± 5.25 lace bugs. There after a steady increase in the feeding potential was observed, showing an eight fold increase from the first to the fourth instar. However, eventhough the fifth instar nymphs consumed 285.00 ± 65.25 lace bugs for 14 days, they failed to moult as adults. Hence, *C. cephalonica* larvae were provided at the fifth instar stage to complete nymphal development. The predator consumed 2.00 ± 0.00 *Corcyra* larvae and successfully moulted as adults (Table 3). Prey consumption of the different instars of *E. plagiatus* on *Corcyra* larvae and *Oecophylla* pupae did not show much variation in the number of prey consumed during the different instars (Table 3).

It was also observed that adults of *E. plagiatus* fed on larvae of *C. cephalonica* consumed 7.00 ± 2.44 lace bugs in the laboratory and 5.00 ± 1.00 lace bugs in field cages and those adults which emerged from cages with *O. smargdina* pupa as prey consumed 9.48 ± 2.09 lace bugs in the laboratory and 6.50 ± 0.75 lace bugs per day on coconut seedlings in field cages (Table 4). In the present study it was observed that the predator consumed more prey in laboratory cages (in test tubes) than in field cages (caged leaves of coconut seedlings). In field cages the predation is probably limited by the prey searching behaviour as compared to the limited confined conditions in the test tubes.

Fecundity and longevity Fecundity of *E. plagiatus* fed on *Corcyra* larvae and *S. typica* are respectively, 263.17 ± 51.59 and 82.67 ± 26.77 eggs (Table 5). *E. plagiatus*

TABLE 3. Prey consumption of nymphal stages of *Euagoras plagiatus* on different hosts in laboratory (No. of prey consumed/instar; Value Mean ± SD)

Nymphal instars	Prey Consumption		
	*S. typica***	*C. cephalonica***	*O. smaragdina****
I	15.67 ± 5.25	4.67 ± 2.22	5.00 ± 1.00
II	32.15 ± 12.38	4.67 ± 2.77	6.00 ± 1.00
III	64.25 ± 14.75	4.21 ± 1.63	5.67 ± 0.58
IV	127.47 ± 38.28	5.00 ± 2.15	7.00 ± 1.00
V	285.00 ± 65.25 (2.00 ± 0.00)*	7.00 ± 1.72	10.33 ± 0.58

* Value in parenthesis denotes the number of *Corcyra* larvae fed during V instar in order to complete development.
** Number of replications - 15
*** Number of replications - 5

TABLE 4. Prey consumption of adult *Euagoras plagiatus* on lace bugs in laboratory and field cage (Value Mean ± SD; Number of replications - Five)

Adults emerged from nymphs reared on	No. of lace bugs consumed/day	
	in laboratory	in field cage
Corcyra cephalonica	7.00 ± 2.44	5.00 ± 1.00
Oecophylla smaragdina	9.48 ± 2.09	6.50 ± 0.75

showed a preoviposition period of 7.00 ± 0.63 and oviposition period of 76.0 ± 18.29 days when fed on *Corcyra* larvae and 5.67 ± 0.58 and 45.0 ± 13.90 days, respectively, when fed on *S. typica*. Egg laying was observed through out the life period. It is also observed that *Corcyra* fed adults lived longer than those fed on *S. typica*. Unfertilised females reared on *Corcyra* laid 243.50 ± 23.23 eggs in the laboratory in an oviposition period of 118.00 ± 20.78 days and these eggs failed to hatch as nymphs. Those which were not provided with prey died within 3–7 days. It was also observed that in the laboratory, the egg hatching was 64% and nymph survival 47% on predators fed on *S. typica* and 83% and 80% respectively, on predators fed on *C. cephalonica* (Table 5).

The mirid *Stethoconus praefectus* is one of the promising predators of *S. typica*. It is present through out the year and is the dominant predator collected from all the locations. Mathen and Kurian (1972) observed that these mirids are capable of consuming 5.20 ± 0.94 lace bugs/day/adult predator. In the present study *E. plagiatus* consumed 9.48 ± 2.09 lace bugs/day/adult predator and the results are comparable to that of *S. praefectus*. *E. plagiatus* could be observed as another potential predator.

Mohanadas (1996) recorded *E. plagiatus* feeding on third instar larvae of *Hyblaea puera* Cramer. Another species of the genus *E. dorycus* Boisd. found preying on both

TABLE 5. Fecundity and longevity of *Euagoras plagiatus* reared on *Corcyra cephalonica* and *Stephanitis typica* (Value Mean ± SD; Number of replications - 15)

Prey consumed	Longevity (in days)		Pre oviposition period (in days)	Oviposition period (in days)	Fecundity per female	Percentage*	
	Male	Female				Hatching	Nymph survival
Stephanitis typica	105.33 ± 12.31	50.50 ± 16.65	5.67 ± 0.58	45.00 ± 13.90	82.67 ± 26.77	64.00 (63/98)	47.00 (7/15)
Corcyra cephalonica	77.67 ± 34.65	83.00 ± 18.10	7.00 ± 0.63	76.00 ± 18.29	263.17 ± 51.59	83.00 (176/213)	80.00 (12/15)

*Actual value in parenthesis

nymphs and adults of the coreid bug, *Amblypelta cocophaga* China on coconut palm in the Solomon islands (Philips, 1940).

Occamus typicus Eggs, nymphs and adults were collected from coconut palms at Thottappally. Adult bugs are brownish green in colour and eggs are cylindrical in shape. Nymphal period is completed in 43–53 days in five instars. The first four instars consumed 10–16, 15–36, 19–38, 50–63 lace bugs/instar, respectively, in the laboratory. *O. typicus* is known as a predator of tea mosquito bug, *Helopeltis antonii* Sign. (Sundararaju, 1984).

While further detailed studies on *O. typicus* and *G. flavipes* are needed, the present studies indicate that *E. plagiatus* could be manipulated for the control of *S. typica*, in coconut ecosystem. It was also observed that *C. cephalonica* larvae and *O. smaragdina* pupae could be used as alternate hosts for laboratory scale mass multiplication of the predator *E. plagiatus* and these could be used for field release for the management of *S. typica*.

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Predatory Potential and Development of the Australian Ladybird Beetle, *Cryptolaemus montrouzieri* Muls. on the Spiralling Whitefly, *Aleurodicus dispersus* Russel

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ABSTRACT: The Australian ladybird beetle *Cryptolaemus montrouzieri* Muls. was found commonly preying on the spiralling whitefly, *Aleurodicus dispersus* Russel in South India. Predatory potential and development of the larvae of *C. montrouzieri* was studied on the nymphs of the spiralling whitefly. The duration of first, second, third and fourth instar larva of *C. montrouzieri* was found to be 4.10, 2.20, 4.50 and 6.40 days respectively when they were fed with *A. dispersus*. The predator had completed its larval development in 17.20 days on the whitefly. The number of whitefly nymphs consumed during first, second, third and fourth larval instar of *C. montrouzieri* averaged to 23.50, 47.85, 74.60 and 149.80 respectively. The predator during the entire larval development consumed a total of 290.75 nymphs. The predator is being evaluated for its potential in the suppression of the spiralling whitefly under field conditions.

KEYWORDS: Predatory potential, Development, *Cryptolaemus montrouzieri*, Spiralling whitefly, *Aleurodicus dispersus*

INTRODUCTION

The Australian ladybird beetle, *Cryptolaemus montrouzieri* Muls. (Coccinellidae, Coleoptera) has often provided spectacular control of heavy infestation of sucking pests in India and elsewhere (Mani and Krishnamoorthy, 1997a). The spiralling whitefly *Aleurodicus dispersus* Russel has been found causing severe damage to several horticultural and agricultural crop plants like guava, mulberry, banana, papaya, chillies, cotton, etc., in South India (David and Regu, 1995; Ranjith *et al.*, 1996; Mani and Krishnamoorthy, 1996). Chemical control is almost impossible since it has got a very wide host range and it is also difficult to control this whitefly with chemicals due to heavy waxy coating and threads over their bodies. Biological control is the other alternative method and efforts must be made to tackle this pest problem. A total

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of nine species of predatory insects were found to attack *A. dispersus* in various plant species in India (Mani and Krishnamoorthy, 1998). Among them, *C. montrouzieri* was observed feeding commonly on spiralling whitefly on many occasions in Coimbatore and Bangalore (Mani and Krishnamoorthy, 1997b). The same predator was also recorded earlier on *A. dispersus* in Hawaii (Paulson and Kumashiro, 1985). The present study was carried out to determine the predatory potential and development of *C. montrouzieri* on *A. dispersus*.

MATERIALS AND METHODS

The culture of *C. montrouzieri* was maintained in the laboratory as outlined by Chacko *et al.* (1978). Twenty newly hatched larvae of *C. montrouzieri* were utilised to conduct the study on the feeding potential and development on the spiralling whitefly. Each predatory larva was considered as replicate. The culture of *A. dispersus* was maintained in the glasshouse on Poinsettia plants. Leaves were collected from the infested plants and brought to the laboratory. After the adult whiteflies were removed, the nymphs were counted in each leaf. A bouquet was prepared with individual leaf and kept in a round bottom plastic jar covered with aerated lid. A single larva was released on the whitefly-infested leaf kept in each plastic jar. Fresh leaves covered with the whitefly nymphs were offered to the predatory larva daily until the pupation of *C. montrouzieri*. Observations were recorded at every 24 hr on the number of nymphs consumed and also the molting of *C. montrouzieri* larva. The number of nymphs consumed by the predatory larva in each instar and also the total number consumed in its larval development was later computed. Development period of each larval instar of *C. montrouzieri* and the total development time were also worked out. During the study period, the maximum and minimum temperature were 28.33°C and 15.93°C respectively. The mean humidity was 64.93% in the morning and 66% in the evening. The data on the prey consumption and larval development was analysed using 'F' test to determine the differences among the larval instars of *C. montrouzieri*.

RESULTS AND DISCUSSION

Incubation period of *C. montrouzieri* ranged from 4 to 5 days. The data on the larval development of *C. montrouzieri* fed with the spiralling whitefly nymphs is presented in Table 1. The duration of first, second, third and fourth larval instar of the predator was 4.10, 2.20, 4.50 and 6.40 days respectively. There were significant differences in the developmental period among different larval instars of *C. montrouzieri* fed with whitefly nymphs. The second instar predatory larva took only 2.20 days where as the fourth instar took 6.40 days. On an average, the predator took 17.20 days to complete its larval development on the nymphs of *A. dispersus*. Similar larval developmental period of *C. montrouzieri* reared on mealybugs was reported by earlier workers in India (Tirumala Rao and David, 1958; Bhat *et al.*, 1981; Mani and Thontadarya, 1987). The pre-pupal and pupal periods of *C. montrouzieri* were 2.5 and 9.0 days respectively. *C.*

AUTHOR INDEX

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